

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁵ : A61K 37/02, C07K 5/00, 7/00, 15/00, 17/00</p>	<p>A1</p>	<p>(11) International Publication Number: WO 95/00161 (43) International Publication Date: 5 January 1995 (05.01.95)</p>
<p>(21) International Application Number: PCT/US94/06837 (22) International Filing Date: 16 June 1994 (16.06.94) (30) Priority Data: 08/079,319 18 June 1993 (18.06.93) US (71) Applicant (for all designated States except US): UNIVERSITY OF CINCINNATI [US/US]; Mall Location 627, Cincinnati, OH 45221-0726 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): BALASUBRAMANIAM, Ambikaipakan [US/US]; 2706 Lawyers Pointe Drive, Cincinnati, OH 45244 (US). (74) Agent: CLARK, Paul, T.; Fish and Richardson, 225 Franklin Street, Boston, MA 02110 (US).</p>		<p>(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.</p>
<p>(54) Title: NEUROPEPTIDE Y ANTAGONISTS AND AGONISTS (57) Abstract The invention discloses analogs which behave as NPY antagonists and agonists; and methods of their use for controlling a biological activity such as appetite and cardiovascular function.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

- 1 -

NEUROPEPTIDE Y ANTAGONISTS AND AGONISTSBackground of the Invention

This invention relates to peptide derivatives
5 which are antagonists or agonists of neuropeptide Y.

Neuropeptide Y (NPY), is a 36-residue peptide characterized by a tyrosine (Y) residue at its N-terminus and a tyrosine amide residue at its C-terminus. The peptide was isolated from porcine brain (Tatemoto *Proc.*
10 *Natl. Acad. Sci. U.S.A.* 79:5485-5489, 1982) and is considered to be a neurotransmitter or neuromodulator widely distributed in the central and peripheral nervous systems (Allen et al., *Neurochem. Int.* 8:1-8, 1986). It is the most abundant peptide present in the mammalian
15 brain and heart (Edvinsson et al., *Trends Pharmacol. Sci.* 8:231-235, 1987; Gu et al., *Histochem. Cytochem.* 32:467-472, 1984), and is among the most potent vasoconstrictor peptides isolated to date (Lundberg et al., *Acta Physiol. Scand.* 121:325-332, 1984). However, NPY elicits several
20 physiological responses by activating specific pre- and post-synaptic receptors. Centrally, NPY is thought to be involved in the regulation of food intake, memory processing and circadian rhythm (Sheikh et al., *FEBS Lett.* 245: 209-214, 1989). In the periphery, NPY seems
25 to function as a transmitter in sympathetic nerves where it interacts with norepinephrine mainly in the regulation of vasculartone (Sheikh et al. *FEBS Lett.* 245:209-214, 1989).

Different structure-activity relationships for NPY
30 analogs in various model systems have indicated that multiple NPY receptor subtypes exist (Michel, *Tips* 12:389-394, 1991). Wahlestedt and coworkers (*Regul. Pept.* 13:307-318, 1986) first suggested the existence of two distinct subtypes of NPY receptors. Post-synaptic
35 (Y1-type) effects could be obtained with the complete NPY

- 2 -

molecule, while pre-synaptic (Y2-type) effects were found elicited by long C-terminal fragments, as well as with the entire NPY molecule. Thus, both Y1 and Y2 receptors exhibit nearly equal affinity to NPY and its homologous peptide, peptide YY, but only the Y2 receptors could bind to shorter carboxyl-terminal fragments including NPY(13-36) as described by Sheikh et al. (*FEBS Lett.* 245:209-214, 1989). However, since NPY receptors in rat cardiac ventricular membranes discriminate between NPY and peptide YY but bind NPY(13-36), it was suggested that this system be classified as a subtype of Y2 or a new class (designated Y3) of receptors as discussed below (Balasubramaniam et al. *Peptides* 11:545-550, 1990).

NPY is also present in high concentrations in a distinct population of nerve fibers innervating the heart and blood vessels (Wharton et al., *Ann. N.Y. Acad. Sci.* 611:133-144, 1990). NPY is now regarded as the predominant peptide present in the cardiovascular system of mammals. This observation has led to numerous studies of the cardiovascular properties of NPY. For example, several investigations have reported that NPY is a potent vasopressor peptide and that it inhibits the coronary blood flow and contractility in isolated perfused hearts (e.g., see Balasubramaniam et al., *Regul. Pept.* 21:289-299, 1988; Allen et al. *Regul. Pept.* 6:247-253, 1983; Rioux et al. *Peptides* 7:27-31, 1986). Furthermore, NPY is also capable of (1) inhibiting the contractile force of isolated cardiac muscles (Balasubramaniam et al. *supra*) and myocytes (Piper et al. *Nuanyn-Scniedberg's Arch. Pharmacol.* 340: 333-337, 1989) and (2) the adenylate cyclase activity and cAMP production by cardiac muscles (Kassis et al., *J. Biol. Chem.* 262: 3429-3431, 1987) and myocytes (Kassis et al. *supra*; Millar et al. *Nuanyn-Scniedberg's Arch. Pharmacol.* 338:426-429, 1989), respectively. Specific receptors of NPY in rat cardiac

- 3 -

5 presence of specific receptors of NPY in rat cardiac
membranes, the Y-3 receptor, was also reported by
visualization with N^α biotinyl-NPY analogs
(Balasubramaniam et al. *Peptides* 11: 1151-1155, 1990

The following table (the abbreviations used are commonly known in the art and are described *infra*) shows the amino acid homology between NPY and PYY:

		5	10	15	20	25	30	35
		-----+-----+-----+-----+-----+-----+-----+-----						
	Human NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITQRQY						
15	Rat NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITQRQY						
	Rabbit NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITQRQY						
	Guinea pig NPY	YPSKPDNPGEDAPAEDMARYYSALRHYINLITQRQY						
	Porcine NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLITQRQY						
		-----+-----+-----+-----+-----+-----+-----+-----						
	Human PYY	YPIKPEAPGEDASPEELNRYYASLRHYLHLVTRQY						
20	Porcine PYY	--A-----S-----						
	Rat PYY	--A-----S-----						

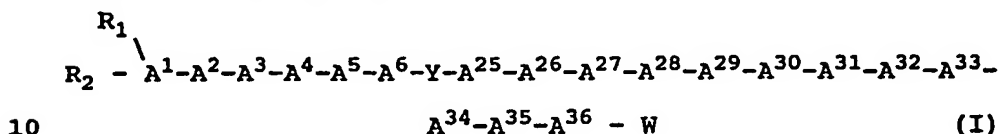
NPY has been implicated in the pathophysiology of a number of diseases including, without limitation, obesity, hypertension and chronic heart failure (CHF) because: (1) hypothalamic NPY levels are elevated in obese rats and decreased in cancer anorectic rats; (2) plasma NPY levels are elevated in CHF and hypertensive patients; (3) negative cardiac inotropic and chronotropic actions; and (4) inhibition of libido and circadian rhythm. Thus, since NPY has been shown to be important for regulating a plurality of physiological events we have set out to design a series of receptor-specific analogs that selectively modulate a variety of biological activities, e.g., appetite and blood pressure activities.

- 4 -

Summary of the Invention

In general, the invention features analogs which behave as NPY antagonists and agonists.

In one aspect, the present invention features
5 compounds having the formula:



wherein each

each R_1 and R_2 , independently, is H, C_1 - C_{12} alkyl
(e.g., methyl), C_6 - C_{18} aryl (e.g., phenyl), C_1 - C_{12}
acyl (e.g., formyl, acetyl, and myristoyl),
15 C_7 - C_{18} aralkyl (e.g., benzyl), or C_7 - C_{18} alkaryl
(e.g., p-methylphenyl);

A^1 is Tyr, or any aromatic amino acid;

A^2 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6C , D-Pal or Asp;

A^3 is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb,
20 Nle, or N-Me-Leu;

A^4 is the D- or L- isomer selected from the group
consisting of Lys, Arg, homo-Arg, diethyl-homo-
Arg, Lys- ϵ -NH-R (where R is H, a branched or
straight chain C_1 - C_{10} alkyl group, or a C_6 - C_{18} aryl
25 group), or Orn;

A^5 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6C , D-Pal, or D-Trp;

A^6 is Gly or is the D- or L- isomer selected from the
group consisting of Asp, Glu, N-Me-Asp, Ala, or
Aoc;

30 Y is A^7 - A^8 - A^9 - A^{10} - A^{11} - A^{12} - A^{13} - A^{14} - A^{15} - A^{16} - A^{17} - A^{18} -
 A^{19} - A^{20} - A^{21} - A^{22} - A^{23} - A^{24} or is absent, where

A^7 is Asn, Ala, Gln, Gly, or N-Me-Asn;

A^8 is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac_6C ,
or D-Pal;

35 A^9 is Gly, N-Me-Gly, Ala, or Trp;

A^{10} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{11} is Asp, Glu, N-Me-Asp, Ala, or Anb;

- 5 -

- A¹² is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A¹³ is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal,
Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal,
Thi, Phe, Bth, Pcp, or N-Me-Ala;
5 A¹⁴ is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal
Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A¹⁵ is Glu, Asp, N-Me-Glu, Ala, or Nva;
A¹⁶ is Asp, Glu, N-Me-Asp, Ala, or Anb;
A¹⁷ is Met, Leu, Ile, Val, Aib, Anb, Nle,
10 or N-Me-Leu;
A¹⁸ is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi,
Phe, Bth, Pcp, or N-Me-Ala;
A¹⁹ is the D- or L- isomer selected from the group
consisting of Lys, Arg, homo-Arg, diethyl-
15 homo-Arg, Lys- ϵ -NH-R (where R is H, a
branched or straight chain C₁-C₁₀ alkyl
group, or a C₆-C₁₈ aryl group), or Orn;
A²⁰ is Tyr, or any aromatic amino acid;
A²¹ is Tyr, or any aromatic amino acid;
20 A²² is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal,
Thi, Phe, Bth, Pcp, or N-Me-Ala,
A²³ is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-
Me-Ala, N-Me-Ser, or N-Me-Thr;
A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
25 A²⁵ is the D- or L- isomer selected from the group
consisting of Lys, Arg, homo-Arg, diethyl-homo-
Arg, Lys- ϵ -NH-R (where R is H, a branched or
straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl
group), or Orn;
30 A²⁶ is the D- or L- isomer selected from the group
consisting of His, Thr, 3-Me-His, β -
pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg,
diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a
branched or straight chain C₁-C₁₀ alkyl group, or
35 a C₆-C₁₈ aryl group), or Orn;

- 6 -

- A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);
- 5 A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;
- A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn or is deleted;
- A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- 10 A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;
- A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr,
- 15 Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotryptophan, or Tcc);
- A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or
- 20 straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;
- A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;
- A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-
- 25 Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- A³⁶ is Tyr, or any aromatic amino acid;
- W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅ ,
- 30 independently, is H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl, (e.g., p-methylphenyl); wherein,
- 35 in formula (I) each bond can represent either a peptide

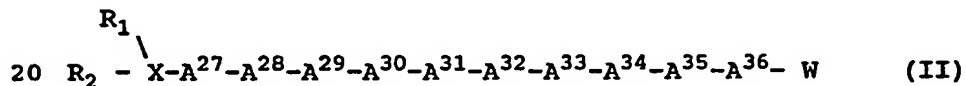
- 7 -

bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

Preferably, said pseudopeptide bond is between
5 amino acid residues A²⁹-A³⁰, A³⁴-A³⁵, and A³⁵-A³⁶.

Preferred compounds formula (I) include those in which A³² is D-Trp, D-Phe, D-Tyr, D-Bip, D-Dip, D-Bth, D-Nal, 2-Cl-Trp, Tcc, Trp, or a pharmaceutically acceptable salt thereof. In yet other preferred embodiments of the
10 invention the compounds of formula (I) include those in which Y (A⁷-A²⁴) is deleted. Preferably, the compound of formula (I) is [D-Trp³²]NPY, cyclo (2/27) Des-AA⁷⁻²⁴[Asp², D-Ala⁶, D-Lys²⁷, D-Trp³²]NPY, Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY, Des-AA⁷⁻²⁴[D-Ala⁵, Gly⁶, D-Trp³²]NPY or
15 Des-AA⁷⁻²⁴[D-Trp⁵, Aoc⁶, D-Trp³²]NPY.

In another aspect, the invention features a compound having the formula:



wherein X is a chain of 0-7 amino acids, inclusive the N-terminal one of which is bonded to each R₁ and R₂; wherein each R₁ and R₂, independently, is

25 H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl (e.g., p-methylphenyl);

A²⁷ is the D- or L- isomer selected from the group
30 consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp,
35 N-Me-Ile, or is deleted;

A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

- 8 -

- A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu, or is deleted;
- A³¹ is Ile, Cys, D-Ala, Leu, Val, Aib, Anb, N-Me-Ile, or is deleted;
- 5 A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g.,
- 10 2-chlorotryptophan, or Tcc);
- A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl
- 15 group), Orn, or is deleted;
- A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;
- A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or
- 20 straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- A³⁶ is Tyr, or any aromatic amino acid;
- W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅, independently, is
- 25 H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl (e.g., p-methylphenyl); wherein, in
- 30 formula (II) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.
- Preferred compounds of formula (II) include those
- 35 where X is A²⁰-A²¹-A²²-A²³-A²⁴-A²⁵-A²⁶ where

- 9 -

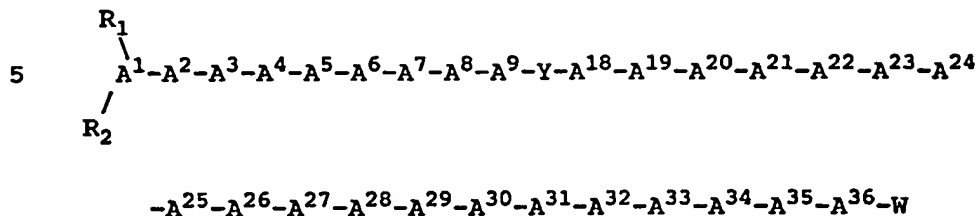
- A²⁰ is Tyr, or any aromatic amino acid;
 A²¹ is Tyr, or any aromatic amino acid;
 A²² is Ser, Thr, N-Me-Ser, or N-Me-Thr;
 A²³ is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-
 5 Me-Ala, N-Me-Ser, or N-Me-Thr;
 A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
 A²⁵ is the D- or L- isomer selected from the group
 consisting of Lys, Arg, homo-Arg,
 diethyl-homo-Arg, Lys- ϵ -NH-R (where R is
 10 H, a branched or straight chain C₁-C₁₀
 alkyl group, or a C₆-C₁₈ aryl group), or
 Orn;
 A²⁶ is the D- or L- isomer selected from the group
 consisting of His, Thr, 3-Me-His, β -
 15 pyrazolylalanine, N-Me-His, Lys, Arg, homo-
 Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is
 H, a branched or straight chain C₁-C₁₀ alkyl
 group, or a C₆-C₁₈ aryl group), or Orn;
 W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅ ,
 20 independently, is
 H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl
 (e.g., phenyl), C₁-C₁₂
 acyl (e.g., formyl, acetyl, and myristoyl),
 C₇-C₁₈ alkaryl; or a pharmaceutically acceptable
 25 salt thereof.

Preferably, said pseudopeptide bond is between amino acid residues A²⁹-A³⁰, A³⁴-A³⁵, and A³⁵-A³⁶.

- Preferably, the compound of formula (II) is [D-Trp²⁸, D-Trp³²]NPY (27-36), (Des-Asn²⁹[D-Trp²⁸, D-
 30 Trp³²]NPY(27-36), Des-Asn²⁹[D-Trp²⁸, D-Trp³², Nva³⁴]NPY(27-
 36), Des-Asn²⁹[Trp²⁸, Trp³², Nva³⁴]NPY(27-36), and [D-
 Trp²⁸, Ant³², Nva³⁴]NPY(27-36), Des-Asn²⁹[D-Trp²⁸, Ant³²,
 Nva³⁴]NPY(27-36), or Des-Asn²⁹, Arg³³[D-Trp²⁸, Ant³²,
 Nva³⁴]NPY(27-36).

- 10 -

In another aspect the invention features a compound having a formula:



10

(III)

wherein a disulfide bond is between A⁷ and A²¹ or is absent; wherein each

each R₁ and R₂, independently, is H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl (e.g., *p*-methylphenyl);

A¹ is Tyr, or any aromatic amino acid;

A² is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal or Asp;

A³ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb, Nle, or N-Me-Leu,

A⁴ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

A⁵ is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal, or D-Trp;

A⁶ is Gly or is the D- or L- isomer selected from the group consisting of Asp, Glu, N-Me-Asp, Ala, or Aoc;

A⁷ is Cys, Glu, Asn, Ala, Gln, Gly, or N-Me-Asn;

A⁸ is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac₆C, or D-Pal;

A⁹ is Gly, N-Me-Gly, Ala, or Trp;

Y is A¹⁰-A¹¹-A¹²-A¹³-A¹⁴-A¹⁵-A¹⁶-A¹⁷ or is absent, where A¹⁰ is Glu, Asp, N-Me-Glu, Ala, or Nva;

- 11 -

- A¹¹ is Asp, Glu, N-Me-Asp, Ala, or Anb;
A¹² is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A¹³ is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal,
Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal,
5 Thi, Phe, Bth, Pcp, or N-Me-Ala Thr;
A¹⁴ is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal
Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A¹⁵ is Glu, Asp, N-Me-Glu, Ala, or Nva;
A¹⁶ is Asp, Glu, N-Me-Asp, Ala, or Anb;
10 A¹⁷ is Met, Leu, Ile, Val, Aib, Anb, Nle,
or N-Me-Leu;
A¹⁸ is, Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth,
Pcp, or N-Me-Ala;
A¹⁹ is the D- or L- isomer selected from the group
15 consisting of Arg, D-homo-Arg, D-diethyl-homo-Arg,
D-Lys- ϵ -NH-R (where R is H, a branched or straight
chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group),
or Orn;
A²⁰ is Tyr, or any aromatic amino acid;
20 A²¹ is Cys, Lys, Tyr, or any aromatic amino acid;
A²² is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe,
Bth, Pcp, or N-Me-Ala;
A²³ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe,
Bth, Pcp, or N-Me-Ala;
25 A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
A²⁵ is the D- or L- isomer selected from the group
consisting of Lys, Arg, homo-Arg, diethyl-homo-
Arg, Lys- ϵ -NH-R (where R is H, a branched or
straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl
30 group), or Orn;
A²⁶ is the D- or L- isomer selected from the group
consisting of His, Thr, 3-Me-His, β -
pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg,
diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a

- 12 -

branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

- 5 A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);
- A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;
- 10 A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn or is deleted;
 A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
 A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;
 A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-
- 15 Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotryptophan, or Tcc);
- A³³ is the D- or L- isomer is selected from the group
- 20 consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, is deleted;
- A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;
- 25 A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 30 A³⁶ is Tyr, or any aromatic amino acid;
 W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅, independently, is H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl),

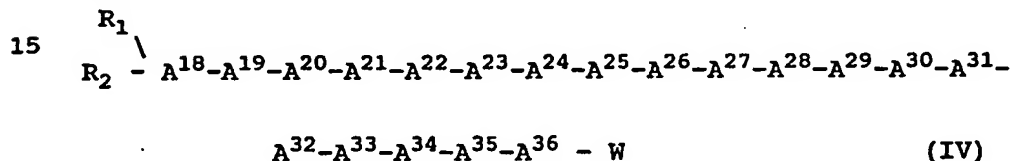
- 13 -

C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl;
 wherein, in formula (III) each bond can represent
 either a peptide bond or a pseudopeptide bond,
 provided that said compound cannot contain more
 5 than 3 pseudopeptide bonds, or a pharmaceutically
 acceptable salt thereof.

Preferably, said pseudopeptide bond is between
 amino acid residues A²⁹-A³⁰, A³⁴-A³⁵, and A³⁵-A³⁶.

Preferably the compound of formula (III) is
 10 cyclo(7/21), Des AA¹⁰⁻¹⁷[Cys⁷, Cys²¹, D-Trp³²]NPY, or
 cyclo(7/21), Des AA¹⁰⁻¹⁷[Glu⁷, Lys²¹, D-Trp³²]NPY.

In another aspect, the invention features a
 compound with pseudopeptide bonds having the formula:



wherein each

20 each R₁ and R₂, independently, is H, C₁-C₁₂ alkyl (e.g.,
 methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂
 acyl (e.g., formyl, acetyl, and myristoyl),
 C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl
 (e.g., p-methylphenyl);

25 A¹⁸ is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth,
 Pcp, or N-Me-Ala;

A¹⁹ is the D- or L- isomer selected from the group
 consisting of Lys, Arg, homo-Arg, diethyl-homo-
 Arg, Lys-ε-NH-R (where R is H, a branched or
 30 straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl
 group), or Orn;

A²⁰ is Tyr, or any aromatic amino acid;

A²¹ is Tyr, or any aromatic amino acid;

A²² is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe,
 35 Bth, Pcp, or N-Me-Ala,

- 14 -

- A²³ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- 5 A²⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 10 A²⁶ is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β -pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 15 A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);
- 20 A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;
- A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;
- A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;
- 25 A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotryptophan, or Tcc);
- 30 A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;
- 35

- 15 -

A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A³⁵ is the D- or L- isomer selected from the group

consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or

5 straight chain C₁-C₁₀ alkyl group, or a C⁶-C₁₈ aryl group), or Orn;

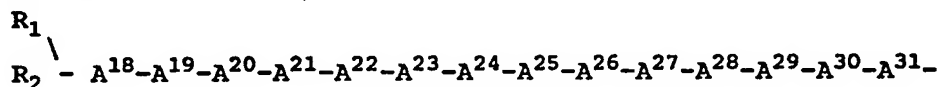
A³⁶ is Tyr, or any aromatic acid;

W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅, independently, is

10 H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl (e.g., p-methylphenyl);

wherein, in formula (IV) each bond can represent either a
15 peptide or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof. In preferred embodiments, the compound contains a pseudopeptide bond between A³⁰ and A³¹; A³¹ and A³²; or A³²
20 and A³³.

In another aspect, the invention features a method of suppressing an NPY mediated physiological response in a tissue other than the heart in a subject comprising administering to said subject a compound having the
25 following formula:



30 $A^{32} - A^{33} - A^{34} - A^{35} - A^{36} - W$

wherein each

each R₁ and R₂, independently, is H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl),

35 C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl

- 16 -

(e.g., *p*-methylphenyl);

A¹⁸ is Ala, Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A¹⁹ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

A²⁰ is Tyr, or any aromatic amino acid;

A²¹ is Tyr, or any aromatic amino acid;

10 A²² is Ser, Thr, N-Me-Ser, or N-Me-Thr;

A²³ is Ala, Ser, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A²⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, D-homo-Arg, D-diethyl-homo-Arg, D-Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

A²⁶ is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β -pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-

- 17 -

Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotryptophan, or Tcc);

5 A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;

10 A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

15 A³⁶ is Tyr, or any aromatic acid;

W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅, independently, is

20 H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl (e.g., p-methylphenyl); wherein, each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or

25 a pharmaceutically acceptable salt thereof. Preferably, said pseudopeptide bond is between amino acid residues A²⁹-A³⁰, A³⁴-A³⁵, and A³⁵-A³⁶. or a pharmaceutically acceptable salt thereof.

30 In preferred embodiments, the method suppresses the activity of the NPY (Y-1) receptor or the NPY (Y-2) receptor.

In another aspect, the invention features a method of suppressing a NPY(Y-1) receptor mediated physiological response in the hypothalamus of a subject comprising

35

- 18 -

administering to said subject the compound of formula (I).

In another aspect, the invention features a method of suppressing the blood pressure of a subject
5 experiencing hypertension which comprises administering to said subject the compound of formula (I).

In another aspect, the invention features a method of suppressing a NPY(Y-3) receptor mediated physiological response in the cardiovascular system of a subject
10 comprising administering to said subject the compound of formula (IV).

In other preferred embodiments, a therapeutically effective amount of a compound of formula (I), (II), (III) or (IV) and a pharmaceutically
15 acceptable carrier substance, e.g., magnesium carbonate or lactose, together form a therapeutic composition capable of suppressing an NPY mediated physiological response. This composition can be in the form a pill, tablet, capsule, liquid, or sustained released tablet for
20 oral administration; or a liquid for nasal administration as drops or spray; or a liquid for intravenous, subcutaneous, parenteral, or intraperitoneal administration.

Another preferred form for administration
25 biodegradable sustained-release composition for intramuscular administration to a subject in need of the composition. Preferably, the composition includes a lipophilic salt and is suitable for administration in the form of an oil emulsion or dispersion to a subject in
30 need of the composition.

In yet another aspect, the invention features methods for suppressing an NPY mediated physiological response in a subject; such methods involve administering one or more of the above mentioned compounds to a subject
35 in a dosage effective to lower blood pressure; to

- 19 -

suppress the appetite; to augment the libido; to stimulate cardiovascular function; on to modulate the circadian rhythm.

In still another aspect, the invention features
5 methods for stimulating an NPY mediated physiological response in a subject; such methods involve administering one or more of the above mentioned compounds to a subject in a dosage effective to increase blood pressure; to increase the appetite; to augment the libido; or to
10 stimulate cardiovascular function.

The symbol A¹, A², A³, and the like; and Tyr, Lys or the like, as found in a peptide sequence herein stands for an amino acid residue, e.g., =N-CH(R)-CO- when it is at the N-terminus, or -NH-CH(R)-CO- when it is at any
15 other position, where R denotes the side chain (or identifying group) of an amino acid or its residue. For example, R is -CH₂COOH for Asp, R is -H for Gly, R is -CH₂OH for Ser, R is -CH₃ for Ala and R is -CH₂CH₂CH₂CH₂NH₂ for Arg. Also, when the amino acid residue is optically
20 active, it is the L-form configuration that is intended unless the D-form is expressly designated. By pseudopeptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon, i.e., CH₂-
25 NH; or less preferably that of C)-NH is replaced with any of CH₂-S, CH₂-O, CH₂-CH₂, CH₂-CO, or CH₂-CH₂. (A pseudopeptide peptide bond is symbolized herein by or "Ψ".) A detailed discussion of the chemistry of pseudopeptide bonds is given in Coy et al. (1988)
30 Tetrahedron 44:835-841.

In other embodiments, the compounds of Formulae (I), (II), (III), or (IV) are cyclic. Preferably, the cyclization is formed by a disulfide or lactam bridge (amide bond). In this disclosure, the disulfide or amide
35 bond which links two residues in a compound of the

- 20 -

invention are formed between the side chain functionalities. That is, between the side-chain carboxyl group of an acidic amino acid residue (e.g., Asp or Glu) and the side chain amino group of a basic amino acid residue (e.g., Lys or Orn), or between the side chain sulfhydryl groups of two Cys. In all formulae set forth herein, the amide or disulfide bond between two residues are not shown. A compound of this invention is also denoted by another format, e.g. cyclo (2/27) Des-AA⁷⁻²⁴[Asp², D-Ala⁶, D-Lys²⁷, D-Trp³²] NPY and cyclo(7/21) Des AA¹⁰⁻¹⁷[Cys⁷, Cys²¹, D-Trp³²]NPY.

Preferred cyclic compounds of the invention are cyclo (2/27) Des AA⁷⁻²⁴[Asp², D-Ala⁶, D-Lys²⁷, D-Trp³²] NPY and cyclo(7/21) Des AA¹⁰⁻¹⁷[Cys⁷, Cys²¹, D-Trp³²]NPY.

In another aspect, the invention features novel dimeric analogs of NPY. The dimer may be formed by either including one compound of Formula I, II, III, or IV and one compound of Formula I, II, III, or IV. In one embodiment, the dimer is formed by utilizing a dicarboxylic acid linker capable of binding to a free amine, either primary or secondary, located within each compound. See R. Vavrek and J. Stewart, Peptides: Structure and Function 381-384 (Pierce Chemical Co. 1983). Examples of suitable dicarboxylic acid linkers are succinic acid, glutamic acid, and phthalic acid. In other embodiments, the dimer is formed by utilizing an amino acid linker capable of binding to a free amine group of one compound and a free carboxylic acid group of the other compound. Preferably, the amino acid linker is a non- α -amino acid. Examples of suitable amino acid linkers are amino-caproic acid and amino-valeric acid. In yet another embodiment, the dimer is formed by disulfide bridge between cysteines located within each compound. See M. Berngtowicz and G. Piatsueda, Peptides: Structure and Function 233-244 (Pierce Chemical Co.

- 21 -

1985); F. Albericio, et al., Peptides 1990 535 (ESCOM 1991).

Preferred dimeric compounds of the invention are Bis(31/31) [Cys³¹, Trp³², Nva³⁴]NPY(27-36), and Bis(31/31)
5 (Cys³¹, Trp³², Nva³⁴]NPY(31-36),

As set forth above and for convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art;
10 but for clarity are listed below. All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right. A short line between two amino acid residues indicates a
15 peptide bond.

Abbreviations (common):

Asp = D = Aspartic Acid
Ala = A = Alanine
20 Arg = R = Arginine
Asn = N = Asparagine
Cys = C = Cysteine
Gly = G = Glycine
Glu = E = Glutamic Acid
25 Gln = Q = Glutamine
His = H = Histidine
Ile = I = Isoleucine
Leu = L = Leucine
Lys = K = Lysine
30 Met = M = Methionine
Phe = F = Phenylalanine
Pro = P = Proline
Ser = S = Serine
Thr = T = Threonine

- 22 -

Trp = W = Tryptophan

Tyr = Y = Tyrosine

Val = V = Valine

Abbreviations (uncommon):

- 5 Aoc = (8-aminooctanoic acid:
Orn = Ornithine
Nal = 2-napthylalanine
Thi = 2-thienylalanine
Pcp = 4-chlorophenylalanine
10 Bth = 3-benzothienylalanine
Bip = 4,4'-biphenylalanine
Tic = tetrahydroisoquinoline-3-carboxylic acid
Aib = aminoisobutyric acid
Anb = α -aminonormalbutyric acid
15 Dip = 2,2-diphenylalanine
Ac₆C = 1-aminocyclohexanecarboxylic acid
D-Pal = β -(3-pyridyl)alanine;
Tcc = tetrahydrocarbolene-carboxylic acid
Nva = norvaline
20 Ant = anthranilic acid
Hyp = hydroxyproline
Nle = norleucine

-
- The compounds of the invention are useful for
- 25 reducing, suppressing or mitigating the effects of NPY.
For example, the compounds of the invention are
especially useful in treating any number of illnesses
that involve eating disorders, cardiovascular function,
alterations in sexual function, as well as disorders of
30 sleep and circadian rhythms (see, e.g., *Harrison's
Principles of Internal Medicine*, McGraw-Hill Inc., New
York, 12th ed.). Specific examples of such disorders,
include without limitation, obesity, anorexia,
hypertension, hypotension, congestive heart failure,

- 23 -

impotence, dyssomnias and rapid time-zone change syndrome. Strategic design of the NPY antagonists, as described herein, allows for the selective antagonism of different classes of NPY receptors, e.g., Y3 cardiac
5 receptors, without adverse interaction with other NPY receptors. The compounds are also useful for stimulating NPY receptor mediated events, e.g., increasing the blood pressure of a subject.

Other features and advantages of the invention
10 will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of Preferred Embodiments

The drawings will first be described.

DRAWINGS

15 Fig. 1 shows the comparison of the effects of D-Trp or D-Trp(CHO) substituted NPY analogs (1.0 μ M) on the isoproterenol stimulated adenylate cyclase activity of rat hypothalamic membranes. Iso, isoproterenol. I., [D-Trp³²] Trp³²]NPY; II, [D-Trp(CHO)³²]NPY; III, [D-Trp³⁴]NPY; IV,
20 [D-Trp(CHO)³⁴]NPY; V, [D-Trp³⁶]NPY; VI, [D-Trp(CHO)³⁶]NPY; a=p, 0.01 compared to isoproterenol; b, not significant compared to isoproterenol.

Fig. 2 shows the displacement of ¹²⁵I-NPY bound to rat hypothalamic membranes by increasing concentrations
25 NPY (●) and [D-Trp³²] NPY (□).

Fig. 3 shows the dose-response effects of increasing concentrations of [D-Trp³²] NPY (□), NPY alone (●); NPY in the presence of 30 (▲) and 300 (■) nM doses of [D-Trp³²] NPY on the isoproterenol stimulated adenylate
30 cyclase activity of rat hypothalamic membranes.

Fig. 4 shows the comparison of the effects of [D-Trp³²]NPY (1.0 μ M) on the inhibition of isoproterenol stimulated adenylate cyclase activity of rat hypothalamic membranes by NPY (100 nM) and serotonin (100 nM). a = p <

- 24 -

0.01 compared to isoproterenol; b, not significant compared to isoproterenol .

Fig. 5 shows the antagonism of NPY induced feeding in rats by [D-Trp³²]NPY.

5 Fig. 6 shows the effects of 1 μ M doses of NPY and its analogs [L-Trp³²] NPY, [D-Trp³²(CHO)] NPY, [D-Nal³²] NPY, [D-Hyp³²] NPY, [(3-1-Tyr²⁷), D-Trp³²] NPY, and [(3-1-Tyr^{27,36}), D-Trp³²] NPY on isoproterenol stimulated adenylate cyclase activity of rat hypothalamic membranes.
10 (iso = isoproterenol); (a = p < 0.005 vs. iso.); (n.s. = not significant).

Fig. 7 shows the effects of increasing concentrations of NPY in the absence (O) and presence (●) of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²] NPY (1 μ M) on the
15 isoproterenol stimulated cAMP production by SK-N-MC cells. Also shown is the effect of increasing concentrations of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY (□) on the isoproterenol stimulated cAMP production by SK-N-MC cells.

20 Fig. 8 shows the effects of increasing concentrations of NPY on the blood pressure of anesthetized rats in the absence (O) and presence (●) of 200 nmol/kg of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY.

Fig. 9 shows the effects of increasing
25 concentrations of NPY (O) and NPY (18-36) (Δ) on the binding of ¹²⁵I-NPY to SK-N-BE2 cells.

Fig. 10 shows the effects of NPY (O), NPY (18-36) (Δ) and NPY in the presence of 1 μ M dose of NPY (18-36) (●) on forskolin stimulated cAMP production by SK-N-BE2
30 cells.

Figs. 11A-11C show the analytical RPLC of [Ψ ³⁰⁻³¹] NPY (18-36) (11A), [Ψ ³²⁻³³] NPY (18-36) (11B), and [Ψ ³³⁻³⁴] NPY (18-36) (11C).

Fig. 12 shows the inhibition of ¹²⁵I-NPY binding
35 to rat cardiac ventricular membrane by NPY (O), NPY

- 25 -

(18-36) (\blacksquare), [$\Psi^{30/31}$] NPY (18-36) (Δ), [$\Psi^{31/32}$] NPY (18-36) (Δ), and [$\Psi^{32/33}$] NPY (18-36) (\square).

Any number of analogs of the invention can be synthesized and tested in one or more of the assays are described below or by methods which are known in the art. We now describe preferred embodiments of the invention.

STRUCTURE

The sequences of naturally occurring NPY are described *supra*. As is easily observed, there is a high degree of amino acid homology between NPY and PYY.

The analogs of the invention have the general formula recited in the Summary of the Invention above. The analogs of the invention are based upon the biologically active full-length molecule (amino acids 1-36) comprising amino acids of NPY and PYY and derivatives thereof; and upon the biologically active subfragments comprising amino acids of NPY and PYY and derivatives thereof.

The analogs of the invention may have one or more modifications to the NPY and PYY sequences (*see above*). For example, the compounds may have one or more of the following modifications which are useful for obtaining selective activity at a NPY receptor: a D-Trp or Aoc or D-Ala in place of one or two or three natural amino acids; or a deletion of several N-terminal amino acids; or the introduction of a pseudopeptide bond instead of a peptide bond between two adjacent amino acids. The analog is capable of acting as a competitive inhibitor of the naturally occurring NPY peptide by binding to the receptor and, by virtue of one of the modifications described *supra* herein, fail to exhibit the biological activity of the naturally occurring peptide. For example, the peptides for which introduction of a pseudopeptide bond between two residues, or the replacement of one or more natural amino acids with a D-

- 26 -

Trp, or the deletion ("des") of the N-terminal residues or internal residues are useful in activity associated NPY activity.

The analogs of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, or pamoic acid, as well as polymeric acids and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric and sulfuric acids.

SYNTHESIS

Peptide Synthesis

The compounds of the present invention, i.e., compounds of formulas (I), (II), (III), (IV), and (V) may be synthesized by any techniques that are known to those skilled in the peptide art. Such techniques are described in, e.g., *Solid Phase Peptide Synthesis*, eds, John M. Stewart and Janis D. Young, Pierce Chemical Company, Rockford, IL, 2nd edition.

The syntheses of the peptides listed in Table 1 and Table 2 were carried out as follows. Peptides were synthesized in an Applied Biosystems model 430A automated instrument, cleaved by hydrogen fluoride, and purified by reversed phase chromatography as described by Balasubramaniam et al. (*Int. J. Pept. Protein Res.* 29:78-83, 1987; *Pept. Res.* 1:32-35, 1988). All synthetic peptides were >98% pure as determined by reverse phase chromatography and had the expected amino acid composition and primary structure. Other analogs can be prepared by making appropriate modifications, within the ability of someone of ordinary skill in this field.

In addition, pseudopeptide bonds may, if desired, may be introduced at various positions, e.g., between amino acid residues 31-32 of NPY(18-36) or between

- 27 -

residues 32-33 of NPY(18-36), or of any peptide as described below. Despite the fact that optically pure Boc-AA-CHO can be obtained in good yields and coupled directly to the α -NH₂ group of the peptide resin by published methods (Sasaki et al., *Peptides* 8:119-121, 1987; Fehrentz et al., *Synthesis* pp.676-678, 1983), this strategy has its limitations because of the possibility of branching at the secondary amine group especially during the synthesis of long peptides with pseudobonds at the C-terminal region. Therefore the utility of several protecting groups, Z, Tos and Z(2-Cl), for capping the secondary amine group in the peptide resin was investigated. Although the reaction of the peptide resin with Z-Cl/Tos-Cl (2 equiv.) & DIEA (4 equiv.) completely blocked the secondary amine, the known lability of Z- during repeated acidolysis to remove Boc group and the apparent resistance of Tos group to HF led us to choose Z(2-Cl) the secondary amine for capping. This is introduced by reacting the peptide resin with Z(2-Cl)-OSU (2 equiv.), HOBT (2 equiv.) and DIEA (4 equiv.) for 10-60 min. The red wine color of ninhydrin with secondary amine turned yellow at the end of capping. This method yielded [$\Psi^{30/31}$]NPY(18-36), [$\Psi^{31/32}$]NPY(18-36) [$\Psi^{32/33}$]NPY(18-36) in greater than 65% yield as judged by analytical HPLC. These peptides not only retained the antagonistic effect, but also exhibited increased affinity (20-220 times) and selectivity for cardiac NPY receptors than NPY(18-36) as discussed below. Integrity of peptides containing pseudobonds were confirmed by mass spectral analysis. Pseudopeptide bond-containing analogs of NPY synthesized by these methods are listed in Table II. Protected amino acid derivatives (Peptide International, Louisville, KY) and peptide synthesis reagents (Applied Biosystems, Foster City, CA) were

- 28 -

obtained commercially and used without further purification.

Examples of the synthesized analogs are:

Formula (I) Compounds

- 5 [D-Trp³²]NPY YPSKPDNPGEDAPAEDLARYYSALRHYINLI [D-Trp]RQRY-NH₂
 [D-Nal³²]NPY YPSKPDNPGEDAPAEDLARYYSALRHYINLI [D-Nal]RQRY-NH₂
 [D-Phe³²]NPY YPSKPDNPGEDAPAEDLARYYSALRHYINLI [D-Phe]RQRY-NH₂
 [D-Hyp³²]NPY YPSKPDNPGEDAPAEDLARYYSALRHYINLI [D-Hyp]RQRY-NH₂
 [L-Trp³²]NPY YPSKPDNPGEDAPAEDLARYYSALRHYINLI [L-Trp]RQRY-NH₂
- 10 Des AA⁷⁻²⁴[D-Trp³²]NPY
 YPSKPD-----RHYINLI [D-Trp]RQRY-NH₂
- Des AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY
 YPSK[D-Ala] [Aoc]-----RHYINLI [D-Trp]RQRY-NH₂
- Des AA⁷⁻²⁴[Aoc⁶, D-Trp³²]NPY
 15 YPSKP [Aoc]-----RHYINLI [D-Trp]RQRY-NH₂

Formula (II) Compounds

- [D-Ala²⁸, D-Trp³²]NPY (27-36) Y [D-Ala]INLI [D-Trp]RQRY-NH₂
- Des-Asn²⁹[D-Trp^{28,32}]NPY (27-36) Y [D-Trp]-LI [D-Trp]RQRY-NH₂

Formula (III) Compounds

- 20 cyclo(7/21), Des AA¹⁰⁻¹⁷[Cys⁷, Cys²¹, D-Trp³²]NPY
 YPSKPDCPG-----ARYCSALRHYINLI [D-Trp]RQRY-NH₂
- cyclo(7/21), Des AA¹⁰⁻¹⁷[Glu⁷, Lys²¹, D-Trp³²]NPY
 YPSKPDEPG-----ARYKSALRHYINLI [D-Trp]RQRY-NH₂

Formula (IV) Compounds

- 25 [30/31]NPY (18-36) -----ARYYSALRHYINLI TRQRY-NH₂
 [31/32]NPY (18-36) -----ARYYSALRHYINLI TRQRY-NH₂
 [32/33]NPY (18-36) -----ARYYSALRHYINLI TRQRY-NH₂

- 29 -

Other analogs of the invention can be prepared as above and tested for their biological activity effectiveness as antagonists or agonists using the methods described below and those commonly known in the art.

FUNCTIONAL ASSAYS

Animals, Cell Lines and Cultures, and Reagents

Any suitable in vivo or in vitro system may be utilized to assay and test the effectiveness of the compounds of the invention. Such assays may employ in vivo methods for evaluating physiological responses, e.g., blood pressure, renovascular function, feeding behavior, or circadian rhythm, or in vivo biochemical systems evaluating receptor binding in a suitable cell line, e.g., SK-N-MC (ATCC#HBT 10) or SK-N-BE(2) (Barnes et al. *In Vitro* 17: 619-631, 1981); or in isolated cells, e.g., cells isolated from the spleen, kidney, heart or brain. A number of in vivo and in vitro biochemical systems known to those skilled in the art are available for testing antagonists to NPY receptors, e.g. the Y-1, Y-2, and Y-3 receptor categories. Described below are assay methods which can be utilized with cell lines such as SK-N-MC and SK-N-BE2 or isolated cardiac membranes which possess the high-affinity NPY receptor sites Y-1, Y-2, and Y-3, respectively. Other systems are also known for evaluating NPY antagonists to the Y-1 receptor, e.g. VSM cells (Sheikh et al., *Am. J. Physiol.* 260: G250-G257, 1991) and HEL cells (Motulsky et al. *Amer. J. Physiol.* 255: E880-E885, 1988); Y-2 receptor, e.g., kidney (Sheikh et al., *Am. J. Physiol* 26:F978-F984), spleen (Lunberg et al., *Eur. J. Pharmacol.* 145:21-29, 1988), dorsal root ganglion (Bleakman et al., *Br. J. Pharmacol.* 103:1781-1789, 1991) and hippocampal cells (Sheikh et al., *J. Biol. Chem.* 265:8304-8310, 1990); and Y-3 receptors, e.g., in cardiac ventricular membranes (Balasubramaniam et al.,

- 30 -

Peptides 11: 545-550, 1990), chromaffin cells, rat gastric mucosa (Michel, M.C., *Trends in Pharmacol. Sci.* 12: 389-394, 1991) and brain stem.

In Vitro Biochemical Assays

- 5 The ability of the compounds of the invention to act as antagonists of NPY can be demonstrated by any number of methods known in the art. For example, the compounds can be shown to compete with iodinated neuropeptide Y for receptors using the methods described
- 10 by Lundberg et al. (*Eur. J. Pharmacol.* 145: 21-29, 1988); Gordon et al. (*J. Neurochemistry* 55:506-513, 1990); Walker et al. (*Mol. Pharmacol.* 34:779-792, 1988); Balasubramaniam et al. (*Peptides* 10:1283-1286, 1989), and others.
- 15 In one working example demonstrating antagonists to Y-1 receptors, rat hypothalamus was isolated and the membranes were prepared for binding and adenylate cyclase studies according to standard methods (Unden et al. 1984. *Eur. J. Biochem* 145: 525-530; Westlind-Danielsson et al.
- 20 1987. *Neurosci. Lett.* 74: 237-242). Displacement studies were performed in a total volume of 0.25 ml 20 mM HEPES buffer, pH 7.4, containing 1% bovine serum albumin, 0.1% bacitracin, 300 μ M PMSF and 5 KIU/ml aprotinin. In a standard assay, 100 μ g of membrane/tube was incubated in
- 25 a shaking water bath at 24° C for 45 min with [125 I-Tyr¹]-NPY (20,000 CPM) as described by Balasubramaniam et al (*Peptides* 11: 545-550, 1990) in the presence of increasing concentrations of NPY (10^{-11} - 10^{-5} M). At the end of incubation, 1.0 ml of iced cold buffer was added,
- 30 centrifuged at 10,000 X g for 10 min, and the supernatant removed by aspiration. The tube containing the pellet was counted for bound radioactivity in a micromedic gamma-counter.

 An example of assaying adenylate cyclase activity

35 of hypothalamic and cerebral cortex membranes is now

- 31 -

described. Adenylate cyclase activity of the hypothalamic and cerebral cortex membranes was determined by incubating 50 μ g of membranes in a total volume of 0.20 ml Tris-HCL 30 mM pH 7.4 buffer containing 150 mM NaCl, 8.25 mM $MgCl_2$, 0.75 mM EGTA, 1.5 theophylline, 20 μ g/ml aprotinin, 100 μ g/ml bacitracin, 1 mg/ml bovine serum albumin, 1 mM ATP, 20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase, 10 μ M isopreternol, 10 μ M GTP, and various concentrations of peptides (0-10 μ M).

10 After incubating the mixture at 35° C for 15 min in a shaking water bath, the reaction was arrested by the addition of 100 μ M EDTA and boiling for 3 min. cAMP was extracted and quantitated by radioimmunoassay. All the points in the binding and adenylate cyclase are the means

15 of at least three parallel experiments performed in duplicate.

In one working example demonstrating antagonists to Y-3 receptors, rat cardiac ventricular membranes and iodination of NPY were prepared according to the method

20 described by Balasubramaniam et al. (*Peptides* 11: 545-550, 1990). Displacement studies were performed in a total volume of 0.25 ml of 20 mM HEPES assay buffer, pH 7.6, containing 2% bovine serum albumin, 100 μ M phenylmethanesulfonyl fluoride, 4 μ g/ml leupeptin, 4 μ g/ml

25 chymostatin, 5 kallikrein-inactivating units/ml aprotinin, and 0.1% bacitracin. In a standard assay, 200 μ g of membrane protein/tube were incubated for 2 h at 18°C in a shaking water bath with 125 I-NPY (40 pM) and increasing concentrations of peptides. At the end of

30 incubation, tubes were vortexed and 150 μ l aliquots transferred into polypropylene tubes containing 250 μ l of ice-cold assay buffer. Unbound 125 I-NPY was separated by centrifugation at 10,000 x g for 10 min followed by aspiration of the supernatant. The tubes containing the

35 pellet were counted for bound radioactivity in a

- 32 -

Micromedic γ counter. The IC_{50} values were used to calculate the equilibrium dissociation constant, K_i for NPY and NPY antagonists using the equation $K_i = IC_{50}/(1 + F/K_d)$, where F and K_i denote the concentration and the dissociation constant of ^{125}I -NPY.

Adenylate cyclase activity was measured by Rosselin et al. (*Biochim. Biophys. Acta* 304:541-551, 1977). Each experiment was carried out in a total volume of 200 μ l solution containing 30 mM Tris-HCl, pH 7.4, 150 mM NaCl, 8.25 mM $MgCl_2$, 0.75 mM EGTA, 1.5 mM theophylline, 20 μ g/ml aprotinin, 100 μ g/ml bacitracin, 1 mg/ml BSA, 1 mM ATP, 20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase, 10 μ M isoproterenol, 10 μ M GTP, and various concentrations of peptides (0-10 μ M). The reaction was initiated by the addition of 50 μ g (50 μ l) of membrane protein. After incubation at 35°C for 10 min. in a shaking water bath, the reaction was terminated by the addition of 100 μ M EDTA and boiling for 3 min. cAMP was extracted and quantitated by radioimmunoassay using a kit obtained from New England Nuclear, Boston, MA.

In Vivo Assays

Any suitable in vivo model system can be used to evaluate the antagonistic properties of the compounds of the invention. Such models, without limitation, include those used to evaluate feeding and memory behavior (Flood et al., *Peptides* 10:963-966), and vasoconstriction and hypertension (Balasubramaniam et al. *Biochim et Biophys Acta* 997: 176-188, 1989).

Thus, in one working example, feeding studies were performed using Sprague Dawley rats (350-450 g) with paraventricular hypothalamic cannulae to investigate effects of NPY analogs (Chance et al. 1989. *Peptides* 10: 1283-1286). Antagonism of NPY induced feeding in rats was by [D-Trp³²]NPY. Groups of rats received intrahypothalamic injections (1 μ l) of artificial CSF or

- 33 -

10 μ g of [D-Trp³²]NPY. Fifteen minutes later CSF-treated rats were injected with CSF (n = 6), 1 μ g of NPY (n = 6) or 10 μ g of [D-Trp³²]NPY (n = 7), while the [D-Trp³²]NPY-treated rats were injected with 1 μ g of NPY (n = 8).

- 5 Rats were provided with a known quantity of rat chow, and after 1 hr the food consumed was determined and corrected for spillage a = p < 0.01 vs. CSF; b, not significant vs. CSF; c = p < 0.01 vs. NPY; d = p < 0.05 vs. NPY.

In another working example blood pressure studies were performed to evaluate the antagonistic properties of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY. The method is as follows, after surgical preparation, three doses of NPY (0.1, 1.0 and 10 nmol/kg) were administered by intravenous push to 7 rats in a randomized order. Each dose was separated by a 20 minute washout period. After obtaining baseline systolic blood pressure (SBP) values, the rats received either 200 nmol/kg of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY (n=5) or 0.9% saline (n=2) prior to each NPY dose. Change in SBP from basal state to maximum SBP observed following NPY was compared between baseline and Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY treatments. The duration of SBP effect of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY was determined in 3 animals by administering 1.0 nmol/kg of NPY every 15 minutes for 75 minutes following a single 200 nmol/kg dose of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY.

RESULTS

We first synthesized a series of full length analogs of NPY substituting either D-Trp or D-Trp(CHO) in the C-terminal receptor binding region at positions 32, 34 and 36. We tested for agonist activity on isoproterenol-stimulated hypothalamic adenylate cyclase activity. Fig. 1 shows that at 1.0 μ M, NPY, [D-Trp³⁴]NPY, [D-Trp³⁶]NPY, and the corresponding formulated D-Trp

- 34 -

analogs inhibited isoproterenol-stimulated hypothalamic adenylate cyclase activity significantly. [D-Trp³²]NPY and its formulated derivative, however, did not exhibit significant inhibitory effect on adenylate cyclase activity at this concentration. In the binding experiments shown in Fig. 2, NPY and [D-Trp³²]NPY inhibited ¹²⁵I-NPY bound to rat hypothalamic membranes in a dose-dependent manner with IC₅₀ values of 0.63 nM and 3.0 nM, respectively. It is this high receptor activity and the complete loss of intrinsic activity that suggests that [D-Trp³²]NPY may be an antagonist of NPY in rat hypothalamus.

The complete loss of intrinsic activity, while retaining high binding potency suggested that [D-Trp³²]NPY may be an antagonist of NPY in hypothalamus. In order to further substantiate this observation, we investigated the inhibitory effect of NPY on rat hypothalamic membrane adenylate cyclase activity both in the absence and presence of [D-Trp³²]NPY. Fig. 3 shows that NPY inhibited isoproterenol stimulated hypothalamic membrane adenylate cyclase activity dose-dependently with an IC₅₀ value 0.18 nM. [D-Trp³²]NPY did not exhibit any inhibitory effect on adenylate cyclase activity. Further, Fig. 3 shows that the presence of 30 and 300 nM [D-Trp³²]NPY shifted the inhibitory dose-response curve of NPY on hypothalamic adenylate cyclase activity to the right increasing that IC₅₀ value to 4.0 nM (K_B = 1.41 nM) and 540. nM (K_B = 1.36 nM), respectively.

To assess the specificity of [D-Trp³²]NPY, we investigated its effect on the inhibitory hypothalamic adenylate cyclase activity of serotonin. Fig. 4 shows that the presence of serotonin (100 nM) significantly (p < 0.01; by repeated measures ANOVA) inhibited the isoproterenol stimulate adenylate cyclase activity both in the absence and presence of [D-Trp³²]NPY (1 μM). The

- 35 -

antagonism at [D-Trp³²]NPY, therefore, was specific to the NPY receptor since the analog exhibited no effect on the inhibitory hypothalamic AC activity of serotonin and, thus, did not act as a global antagonist.

5 Since hypothalamic NPY has been shown to elicit a feeding response, we also investigated the effect of [D-Trp³²]NPY on NPY induced feeding in freely moving rats. Fig. 5 shows that intrahypothalamic injection of NPY (1 μ g) significantly ($p < 0.01$) stimulated the cumulative
10 food intake as compared to vehicle (artificial cerebrospinal fluid) treatment over 1 hr. On the other hand, [D-Trp³²]NPY (1 μ g) did not stimulate feeding significantly over this period, nor did it attenuate NPY (1 μ g) - induced feeding at this concentration. 10 μ g of
15 [D-Trp³²]NPY also did not exhibit significant effect on feeding, and at this dose significantly ($p < 0.05$) attenuated the 1 hr. cumulative food intake induced by 1 μ g of NPY. All of these observations suggest that D-Trp³² is a specific and competitive antagonist at NPY in rat
20 hypothalamus in both in vitro and in vivo models.

 In order to improve the potency and/or selectivity, several analogs were synthesized substituting the residue at 32 with various amino acids, e.g., D-Nal, D-Phe, D-Hyp, or L-Trp (Fig. 6). However,
25 these analogs exhibited agonistic activity which suggests there are strict structural requirements to induce antagonistic properties to NPY. Although it is generally believed that the NPY effects on blood pressure and feeding are mediated by the Y-1 receptor subtype, it is
30 possible that NPY analogs which elicit pressor effects have no orexigenic effects. Thus, [D-Trp³²]NPY is useful not only to elucidate the receptor subtypes mediating NPY effects on hypothalamus, but also to determine whether feeding and pressor effects are mediated by the Y-1
35 receptors.

- 36 -

Next, the relative binding affinities of various compounds having formula (I) were investigated using SK-N-MC (Y-1) and SK-N-BE2(Y-2) shown in Table I. These studies led to the development of two truncated peptide analogs, Des-AA⁷⁻²⁴[Aoc⁶, D-Trp³²]NPY and Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY, which did not inhibit the cAMP production by SK-N-MC cells (see Table I). However, Des-AA⁷⁻²⁴[Aoc⁶, D-Trp³²]NPY exhibited poor affinity to Y-1 receptors (Table I), and therefore, failed to antagonize the inhibitory effects of NPY on SK-N-MC cAMP production. On the other hand, Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY, surprisingly exhibited moderate affinity (Table I), and its presence (1.0 μ M) shifted the inhibitory dose-response curve of NPY on SK-N-MC cAMP production parallel to the right (Fig. 7). These observations confirm that Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY is a competitive antagonist of NPY in Y-1 receptors.

To investigate whether these compounds retained antagonistic activity within an *in vivo* model, we tested the effects on NPY-induced anorectic rats. Fig. 8 shows that NPY doses of 0.1, 1.0 and 10.0 nmol/kg, during baseline, increased systolic blood pressure (SBP) by 8 \pm 7, .26 \pm 6 and 37 \pm 7 mmHg respectively. Following administration of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY, NPY doses of 0.1, 1.0 and 10.0 nmol/kg increased SBP by 4 \pm 5, 9 \pm 5 and 29 \pm 17 mmHg respectively. The change in SBP during Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY was significantly different than baseline values ($p = 0.0002$ at the 1.0 nmol/kg NPY doses, but not at the 0.1 or 10 nmol/kg doses. Changes in SBP in control rats receiving saline were not significantly different than baseline values at all NPY doses. The duration of effect of the antagonist ranged between 30-75 minutes. This result demonstrates that Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY is effective in attenuating NPY induced vasoconstriction in

- 37 -

vivo. Its ability to only affect SBP at the middle NPY dose and the finding that Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY can inhibit the binding of ¹²⁵I-NPY to SK-N-MC cells, suggests that Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY 5 competitively antagonizes NPY induced hypertension.

In addition, further truncation and deletion of Des-AA⁷⁻²⁴[D-Ala⁵, Aoc⁶, D-Trp³²]NPY resulted in the development of three analogs (Table I). Although these analogs did not bind to Y-2 receptors, both [D-Ala²⁸], D- 10 Trp³²]NPY(27-36) and [Bip²⁷, D-Ala²⁸, D-Trp³²]NPY(27-36) also exhibited poor affinity to Y-1 receptor. However, Des-Asn²⁹[D-Trp^{28,32}]NPY(27-36) bound with moderate potency to Y-1 receptors, and also did not exhibit any intrinsic activity on isoproterenol stimulated cAMP 15 production by SK-N-MC cells. These observations suggest that Des-Asn²⁹[D-Trp^{28,32}]NPY(27-36) or its analogs will prove useful for the development low molecular weight selective antagonist compounds for Y-1 receptors.

TABLE I

20	Peptides	IC ₅₀ (nM) for the inhibition of ¹²⁵ I-NPY binding to	
		SK-N-MC (Y-1)	SK-N-BE2 (Y-2)
	NPY	1.3	0.1
25	[D-Trp ³²]NPY	1000	0.63
	Des-AA ⁷⁻²⁴ [Aoc ⁶ , D-Trp ³²]NPY	3900	10.0
	Des-AA ⁷⁻²⁴ [D-Ala ⁵ , Aoc ⁶ , D-Trp ³²]NPY	100	1.0
	[D-Ala ²⁸ , D-Trp ³²]NPY(27-36)	630	N.I.
30	[Bip ²⁷ , D-Ala ²⁸ , D-Trp ³²]NPY(27-36)	1300	N.I.
	Des-Asn ²⁹ [D-Trp ^{28,32}]NPY(27-36)	170	N.I.

35 N.I.: no inhibition even at 10,000 nM

- 38 -

The analogs of the invention may also be assayed and tested for NPY receptor Y-2 activity using the methods described *supra*. Thus, a compound, e.g., [D-Trp³²]NPY, can be assayed for antagonism using any Y-2 receptor bearing cell, e.g., the SK-N-BE2 cell line, or such cells found in the spleen, kidney, hippocampus or dorsal root ganglion.

Towards developing selective agonists and antagonists of Y-2 receptors, we tested a number of compounds using SK-N-BE2 cell lines. These studies demonstrated that NPY(18-36), previously shown to be an antagonist of NPY in rat cardiac membranes bearing Y-3 receptors, antagonizes the inhibitory effect on the cAMP production of SK-N-BE2 cells bearing Y-2 receptor subtypes as shown in Figures 9 and 10.

NPY RECEPTOR (Y-3 SUBTYPE)

Next, we investigated the effect of introducing a pseudopeptide bond to NPY(18-36). Table II shows the results for the increased affinity and selectivity of pseudopeptide analogs of NPY(18-36) for Y-3 receptors. The introduction of pseudobonds (-CH₂NH-) at positions 31-32 or 32-33 of NPY(18-36) was found to substantially increase Y-3 receptor affinity (see Table 2). Subsequent experiments revealed that all these analogs retain their antagonistic properties. Furthermore, [$\psi^{30/31}$]NPY(18-36) and [$\psi^{31/32}$]NPY(18-36) and [$\psi^{32/33}$]NPY(18-36) analogs exhibit lower affinity to Y-1 and Y-2 subtypes than NPY(18-36) (Table II). Thus, introduction of pseudobonds at 32-33 and 31-32 also increases their selectivity for Y-3 receptors.

- 39 -

TABLE II

PEPTIDES	IC ₅₀ (nM) for the inhibition of ¹²⁵ I-NPY binding to:		
	Y-3 (CARDIAC)	Y-2 (SK-N-BE2)	Y-1 (SK-N-MC)
NPY	0.20	0.1	1.3
NPY(18-36)	126	3.00	251
[Ψ ³²⁻³³]NPY(18-36)	0.56	158	1585
[Ψ ³¹⁻³²]NPY(18-36)	1.00	562	1995
[Ψ ³⁰⁻³¹]NPY(18-36)	6.00	281	N.D.

Ψ, -CH₂NH-; N.D., not determined.

EXAMPLES

This invention is further illustrated by the following nonlimiting examples.

EXAMPLE 1

Synthesis of [D-Ala⁵, Aoc⁶, D-Trp³²]NPY

Peptide Synthesis -- MBHA resin (0.45 mM NH₂ group) was placed in a reaction vessel of the Applied Bioscience (ABI) 430A automated instrument and amino acid derivatives were coupled automatically using the standard program provided by the manufacturer modified to incorporate a double coupling procedure. All amino acids were coupled using 2.2 equivalents of preformed symmetrical anhydrides. Arg, Asn and Gln, however, were coupled as preformed 1-HOBT esters (4.4 equal.) to avoid deamidation or lactam formation. At the end of the

- 40 -

synthesis N- α -Boc-group was removed and peptide resin (~1g) was treated with HF as described below.

In the reaction vessel 1.0 g peptide resin, 0.8 g p-cresol, 0.2g thiocresol, 0.8 ml $(\text{CH}_3)_2$ and 5 ml HF were stirred for 40 min of reaction and an additional 60 min. of HF evacuation. During these procedures temperature of reaction vessel was kept between 0°C - -4°C. Then the peptide resin was transferred into a fitted filter funnel in Et_2O and washed with excess of Et_2O . Free peptide was extracted with 30% HOAc (2x15ml). Peptide solution was diluted to 10% HOAc (60ml H_2O) and lyophilized. 390 mg crude peptide was obtained from this procedure.

EXAMPLE 2

15 Synthesis of [D-Trp³²] NPY

Peptide synthesis was performed as described above. Cleavage by HF was as follows: in a reaction vessel 1.0g peptide resin, 0.8 ml $(\text{CH}_3)_2\text{S}$, 0.8g p-cresol, 0.2g p-thiocresol and 5ml HF were stirred for 40 min of reaction in temperature between 0°C - -4°C. After that HF was evacuated in 60. Temperature was still kept below 0°C. The peptide resin was transferred into fitted filter funnel and washed with excess of Et_2O . The peptide resin extracted with 30ml 30% HOAc. Peptide solution was diluted to 10% HOAc with 60ml H_2O and protein lyophilized. Total weight of crude peptide: 190mg.

EXAMPLE 3

Synthesis of Cyclo(7/21), Des-AA¹⁰⁻¹⁷[Cys^{7,21},D-Trp³²] NPY

Peptide synthesis was as described above using an Automated ABI 430A synthesizer. The free peptide was obtained by treating the protected peptide resin (1.0g) with HF (10 ml) containing dimethyl sulfide (0.8 ml), p-cresol (0.2g) for 1 h at -2 to -4 C. The residue was

- 41 -

transferred to a fitted filter funnel with diethyl ether, washed repeatedly with diethyl ether, and the peptide extracted with 10% HOAC(2X 15 ml) and lyophilized. The crude peptide (100mg) thus obtained was dissolved in 6M guanidine HCL (6 ml) diluted with 500 ml of distilled water and the pH adjusted to 8 with ammonia. A solution of potassium ferricyanide (1% w/v) was gradually added with constant stirring until a yellow color persisted. After stirring for an additional 30 min., the pH of the solution was adjusted to 5 with acetic acid and the solution stirred with an anion exchange resin (AG-3, Cl-form, 10g wet weight) for 30 min, passed through a 0.45 microns filter, and pumped into a semipreparative column (250X10 mm), washed with 0.1%TFA-H₂O until a flat base line was obtained. The column containing the peptide was then subjected to gradient elution as described for NPY, and the purified peptide was characterized by amino acid and mass spectral analysis.

EXAMPLE 4**20 Synthesis of Cyclo(7/21), Des-AA¹⁰⁻¹⁷[Glu⁷, Lys²¹, D-Trp³²]NPY**

The synthesis of this peptide was accomplished using the general strategy described for NPY except for the following: After coupling BocGlu(OFM) at position 7, the side chain protecting groups, ϵ -Fmoc group at Lys²¹ and the γ ORm of Glu⁷ were removed by removing the peptide resin with 20% piperidine-DMF. After repeated washings with DMF, the ϵ -NH₂ group of Lys²¹ was coupled to γ -COOH of Glu⁷ by stirring the peptide resin with BOP-HOBT-DIPEA (1:1:3) in DMF (20 ml) overnight, and if cyclization is not complete as judged by the standard ninhydrin test the procedure was repeated until complete cyclization has occurred. The synthesis was then continued in the

- 42 -

automated mode, and the free peptide was obtained by the standard HF method described in Example 3.

Example 5

Synthesis of [ψ 32/33]NPY (18-36)

5 Standard techniques, as described above, were employed for the solid phase synthesis of the carboxy terminal portion of cardiac receptor antagonist, NPY [ψ 32/33]NPY (18-36), up to the point at which introduction of the pseudopeptide bond was desired. The pseudopeptide
10 bond was then introduced in the analog according to the method of Sasaki et al. (*Peptides* 8:119-121, 1986), with Boc as the protecting group for the primary amine.

 The resulting N- α -Boc-peptide-resin with the pseudopeptide bond (0.25 mmol) was swollen in DMF (10 ml)
15 for 10 min in a two-necked R.B. flask fitted with a drying tube. This was followed by the addition of diisopropylethyl amine (1.0 mmol), HOBt (0.5 mmol) and Z(2-Cl)OSU (0.5 mmol). HOBt enhances the coupling of Z(2-Cl) to the secondary amino group of the pseudopeptide
20 bond. The reaction mixture was stirred at room temperature until the Kaiser's ninhydrin test gave a yellow color indicating that the secondary amine had been blocked. The peptide resin was returned to the reaction vessel of the automated peptide synthesizer and the rest
25 of the sequence was assembled automatically. The free peptide was obtained by the standard cleavage conditions and purified by reverse phase chromatography.

USE

 Because NPY is a potent vasoconstrictor and or
30 orexigenic agent, as well as an inhibitor of libido and effector of circulation rhythm, it is likely that the administration of one or more compounds of the invention may suppress or inhibit the deleterious effects of NPY.

- 43 -

Therefore, the NPY antagonists of the invention are suitable for the treatment of any number of diseases related to cardiovascular function (e.g., congestive heart failure or hypertension), obesity, anorexia, blood pressure, asthma, pulmonary hypertension, renal hypertension, memory retention, sexual dysfunction (e.g. impotence), and disorders involving sleep and circadian rhythms. For example, the compounds of formula (I), (II), (III) are useful for treating for controlling feeding disorders and blood pressure; the compounds of formula (IV) are useful for treating any number of heart ailments, e.g., chronic heart failure, as well as promoting recovery from ischemia since the compounds are expected to enhance myocardium contraction; and the compounds of formula (IV) are useful for controlling NPY actions mediated by Y-2 receptor subtypes, e.g., for controlling the effects of NPY on renal blood flow, glomerular filtration rate, natriuresis and renin secretion.

Thus to treat the above disorders, the appropriate NPY antagonist is administered as a therapeutic preparation (as described below) in accordance with the condition to be treated. In the practice of the method of the present invention, an effective amount of an NPY antagonist, e.g., Ψ^{30-31} NPY(18-36), is administered via any of the usual and acceptable methods known in the art, either singly or in combination with another compound or compounds of the present invention. These compounds or compositions can thus be administered orally, sublingually, parenterally (e.g., intramuscularly, intravenously, subcutaneously, or intradermally) or by inhalation, and in the form of either solid, liquid or gaseous dosage, including tablets and suspensions. The administration can be conducted in a single unit dosage

- 44 -

form with continuous therapy or in a single dose therapy
ad libitum.

The dose of the compound of the present invention
for treating the above-mentioned disorders varies
5 depending upon the manner of administration, the age and
the body weight of the subject, and the condition of the
subject to be treated, and ultimately will be decided by
the attending physician or veterinarian. Such amount of
the active compound as determined by the attending
10 physician or veterinarian is referred to herein as a
"therapeutically effective amount". Thus, a typical
administration is oral administration or parenteral
administration. The daily dose in the case of oral
administration is typically in the range of 0.1 to 100
15 mg/kg body weight, and the daily dose in the case of
parenteral administration is typically in the range of
0.001 to 50 mg/kg body weight.

To be effective for the prevention or treatment of
the above-mentioned disorders it is important that the
20 therapeutic agents be relatively non-toxic, non-antigenic
and non-irritating at the levels in actual use.

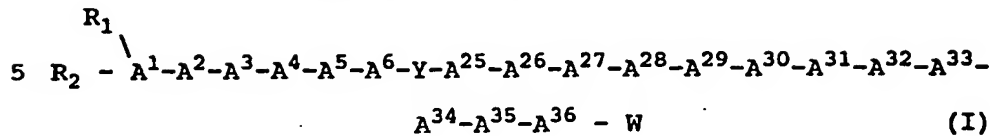
It is understood that the examples and embodiments
described herein are for illustrative purposes only and
that various modifications or changes in light thereof
25 will be suggested to persons skilled in the art and are
to be included within the spirit and purview of this
application and scope of the appended claims.

Other embodiments are within the following claims.

- 45 -

CLAIMS

1. A compound having the formula:



wherein

- each R_1 and R_2 , independently, is H, C_1 - C_{12} alkyl,
 10 C_6 - C_{18} aryl, C_1 - C_{12} acyl,
 C_7 - C_{18} aralkyl, or C_7 - C_{18} alkaryl;
 A^1 is Tyr, or any aromatic amino acid;
 A^2 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6C , D-Pal or Asp;
 A^3 is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb,
 15 Nle, or N-Me-Leu;
 A^4 is the D- or L- isomer selected from the group
 consisting of Lys, Arg, homo-Arg, diethyl-homo-
 Arg, Lys- ϵ -NH-R (where R is H, a branched or
 straight chain C_1 - C_{10} alkyl group, or a C_6 - C_{18} aryl
 20 group), or Orn;
 A^5 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6C , D-Pal, or D-Trp;
 A^6 is Gly or is the D- or L- isomer selected from the
 group consisting of Asp, Glu, N-Me-Asp, Ala, or
 Aoc;
 25 Y is A^7 - A^8 - A^9 - A^{10} - A^{11} - A^{12} - A^{13} - A^{14} - A^{15} - A^{16} - A^{17} - A^{18} -
 A^{19} - A^{20} - A^{21} - A^{22} - A^{23} - A^{24} or is absent, where
 A^7 is Asn, Ala, Gln, Gly, or N-Me-Asn;
 A^8 is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac_6C ,
 or D-Pal;
 30 A^9 is Gly, N-Me-Gly, Ala, or Trp;
 A^{10} is Glu, Asp, N-Me-Glu, Ala, or Nva;
 A^{11} is Asp, Glu, N-Me-Asp, Ala, or Anb;
 A^{12} is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
 A^{13} is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6C , D-Pal,
 35 Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal,
 Thi, Phe, Bth, Pcp, N-Me-Ala, or Thr;

- 46 -

- A¹⁴ is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- 5 A¹⁵ is Glu, Asp, N-Me-Glu, Ala, or Nva;
A¹⁶ is Asp, Glu, N-Me-Asp, Ala, or Anb;
A¹⁷ is Met, Leu, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;
- 10 A¹⁸ is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A¹⁹ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 15 A²⁰ is Tyr, or any aromatic amino acid;
A²¹ is Tyr, any aromatic amino acid;
A²² is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- 20 A²³ is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, N-Me-Ser, or N-Me-Thr;
A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- 25 A²⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 30 A²⁶ is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β -pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

- 47 -

- A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;
- 5 A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;
- A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;
- A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;
- 10 A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;
- 15 A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;
- 20 A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;
- A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 25 A³⁶ is Tyr, or any aromatic amino acid;
- W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅, independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl; wherein, in formula (I) each bond can
- 30 represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

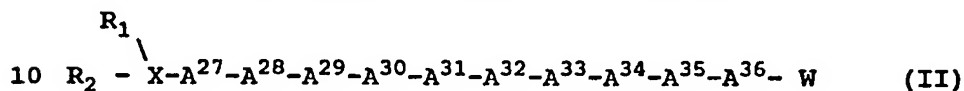
- 48 -

2. The compound of claim 1, wherein Y (A⁷-A²⁴) is absent.

3. The compound of claim 2, wherein said compound has the formula Des AA⁷⁻²⁴, Aoc⁶ D-Trp³²] NPY.

5 4. The compound of claim 2, wherein said compound has the formula Des AA⁷⁻²⁴ [D-Ala⁵, Aoc⁶, D-Trp³²] NPY.

5. A compound having the formula:



wherein X is a chain of 0-7 amino acids, inclusive, the N-terminal one of which is bonded to each R₁ and R₂;

15 wherein each R₁ and R₂, independently, is

each H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl;

20 A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;

A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A²⁹ is Asn, Ala, Gln, Gly, or N-Me-Asn, or is deleted;

25 A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu, or is deleted;

A³¹ is Ile, Cys, D-Ala, Leu, Val, Aib, Anb, or N-Me-Ile, or is deleted;

30 A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;

- 49 -

- A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;
- 5 A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;
- A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 10 A³⁶ is Tyr, or any aromatic amino acid;
- W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅, independently, is
- 15 H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ alkaryl or C₇-C₁₈ alkaryl; wherein, in formula (II) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a
- 20 pharmaceutically acceptable salt thereof.

6. The compound of claim 5, where X is A²⁰-A²¹-A²²-A²³-A²⁴-A²⁵-A²⁶ wherein
- A²⁰ is Tyr, or any aromatic amino acid;
- A²¹ is Tyr, or any aromatic amino acid;
- 25 A²² is Ser, Thr, N-Me-Ser, N-Me-Thr;
- A²³ is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, N-Me-Ser, or N-Me-Thr;
- A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- A²⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 30

- 50 -

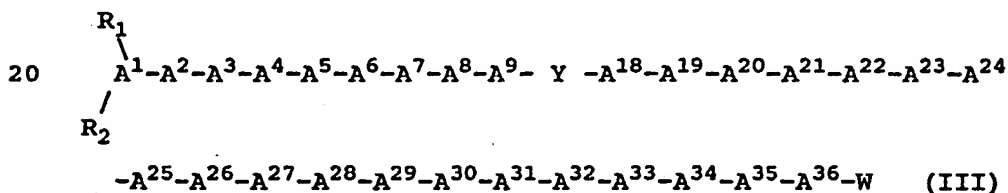
A²⁶ is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β -pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

W is -OH, -N-R₃R₄, or OR₅ (where each R₃, R₄, and R₅, independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl; or a pharmaceutically acceptable salt thereof.

7. The compound of claim 5 having the formula [D-Ala²⁸, D-Trp³²]NPY (27-36).

8. The compound of claim 5, having the formula Des-Asn²⁹ [D-Trp^{28,32}]NPY(27-36).

9. A compound having the formula:



wherein a disulfide bond is between A⁷ and A²¹ or is absent; wherein each R₁ and R₂, independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl;

A¹ is Tyr, or any aromatic amino acid;
 A² is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal or Asp;
 A³ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb, Nle, or N-Me-Leu

- 51 -

- A⁴ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 5 A⁵ is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal, or D-Trp;
- A⁶ is Gly or is the D- or L- isomer selected from the group consisting of Asp, Glu, N-Me-Asp, Ala, or Aoc;
- 10 A⁷ is Cys, Glu, Asn, Ala, Gln, Gly, or N-Me-Asn;
- A⁸ is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac₆C, or D-Pal;
- A⁹ is Gly, N-Me-Gly, Ala, or Trp;
- Y is A¹⁰-A¹¹-A¹²-A¹³-A¹⁴-A¹⁵-A¹⁶-A¹⁷ or is absent, where
- 15 A¹⁰ is Glu, Asp, N-Me-Glu, Ala, or Nva;
- A¹¹ is Asp, Glu, N-Me-Asp, Ala, or Anb;
- A¹² is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- A¹³ is Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal, Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal,
- 20 Thi, Phe, Bth, Pcp, N-Me-Ala, or Thr;
- A¹⁴ is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac₆C, D-Pal, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- A¹⁵ is Glu, Asp, N-Me-Glu, Ala, or Nva;
- A¹⁶ is Asp, Glu, N-Me-Asp, Ala, or Anb;
- 25 A¹⁷ is Met, Leu, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;
- A¹⁸ is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- A¹⁹ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 30

- 52 -

- A²⁰ is Tyr, or any aromatic amino acid;
A²¹ is Cys, Lys, Tyr, or any aromatic amino acid;
A²² is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe,
Bth, Pcp, or N-Me-Ala;
5 A²³ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe,
Bth, Pcp, or N-Me-Ala;
A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
A²⁵ is the D- or L- isomer selected from the group
consisting of Lys, Arg, homo-Arg, diethyl-homo-
10 Arg, Lys- ϵ -NH-R (where R is H, a branched or
straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl
group), or Orn;
A²⁶ is the D- or L- isomer selected from the group
consisting of His, Thr, 3-Me-His, β -
15 pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg,
diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a
branched or straight chain C₁-C₁₀ alkyl group, or
a C₆-C₁₈ aryl group), or Orn;
A²⁷ is the D- or L- isomer selected from the group
20 consisting of any aromatic amino acid, Lys, or a
tethered amino acid with an indole ring;
A²⁸ is Aib or is the D- or L- isomer selected from the
group consisting of Ile, Leu, Val, Anb, Trp,
N-Me-Ile, or is deleted;
25 A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn, or deleted;
A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;
A³² is the D- or L- isomer selected from the group
consisting of any aromatic amino acid except L-
30 Tyr, a tethered amino acid with an indole ring,
Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala,
D-Hyp, or any Trp derivative;
A³³ is the D- or L- isomer selected from the group
consisting of Lys, Arg, homo-Arg, diethyl-homo-
35 Arg, Lys- ϵ -NH-R (where R is H, a branched or

- 53 -

straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;

A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A³⁵ is the D- or L- isomer selected from the group
 5 consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;

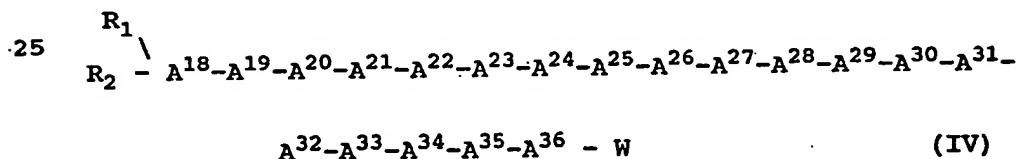
A³⁶ is Tyr, or any aromatic amino acid;

10 W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅ , independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl; wherein, in formula (III) each bond can represent either a peptide
 15 bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

10. The compound of claim 9, having the formula
 cyclo(7/21), Des AA¹⁰⁻¹⁷[Cys⁷, Cys²¹, D-Trp³²]NPY.

20 11. The compound of claim 9, having the formula
 cyclo(7/21), Des AA¹⁰⁻¹⁷[Glu⁷, Lys²¹, D-Trp³²]NPY.

12. A compound with pseudopeptide bonds having the formula:



wherein

30 each R₁ and R₂, independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl;

- 54 -

- A¹⁸ is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- A¹⁹ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 5 A²⁰ is Tyr, or any aromatic amino acid;
- A²¹ is Tyr, or any aromatic amino acid;
- 10 A²² is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala
- A²³ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
- A²⁴ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- 15 A²⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 20 A²⁶ is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β -pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- 25 A²⁷ is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;
- A²⁸ is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;
- 30 A²⁹ is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;
- A³⁰ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- A³¹ is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

- 55 -

- A³² is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;
- 5 A³³ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), Orn, or is deleted;
- 10 A³⁴ is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;
- A³⁵ is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or
- 15 straight chain C₁-C₁₀ alkyl group, or a C₆-C₁₈ aryl group), or Orn;
- A³⁶ is Tyr, or any aromatic acid;
- W is -OH, -N-R₃R₄, or OR₅ (where R₃, R₄, and R₅ , independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ acyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl;
- 20 wherein, in formula (IV) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 2 pseudopeptide bonds, or a pharmaceutically
- 25 acceptable salt thereof.

13. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A²⁹ and A³⁰.

14. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A³⁰ and A³¹.

30 15. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A³¹ and A³².

- 56 -

16. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A³² and A³³.
17. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A³⁴ and A³⁵.
- 5 18. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A³⁵ and A³⁶.
19. A dimeric compound comprising one compound from either claims 1, 5, 9, or 12 and one compound from either claims 1, 5, 9, or 12, wherein said dimer is
10 formed by either an amide bond or a disulfide bridge between the two compounds.

1/12

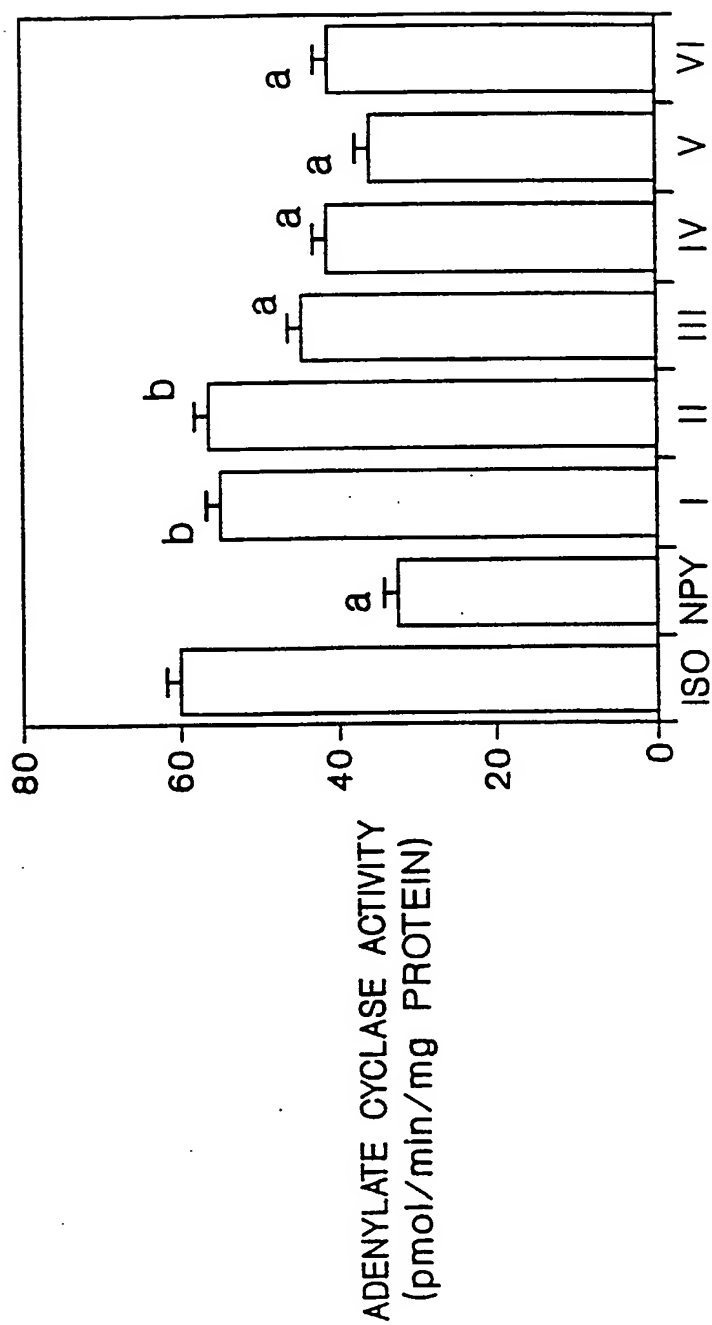


FIG. 1

2/12

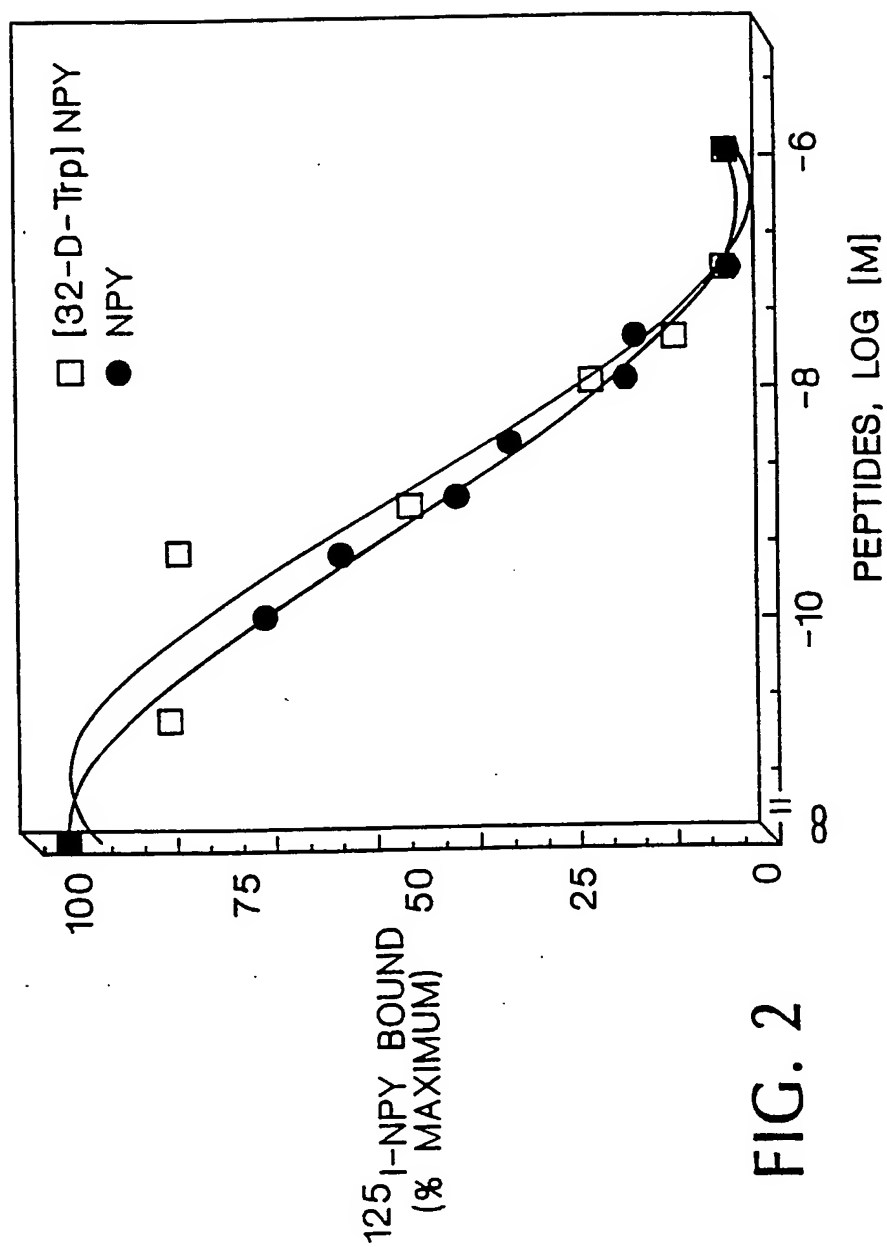


FIG. 2

3/12

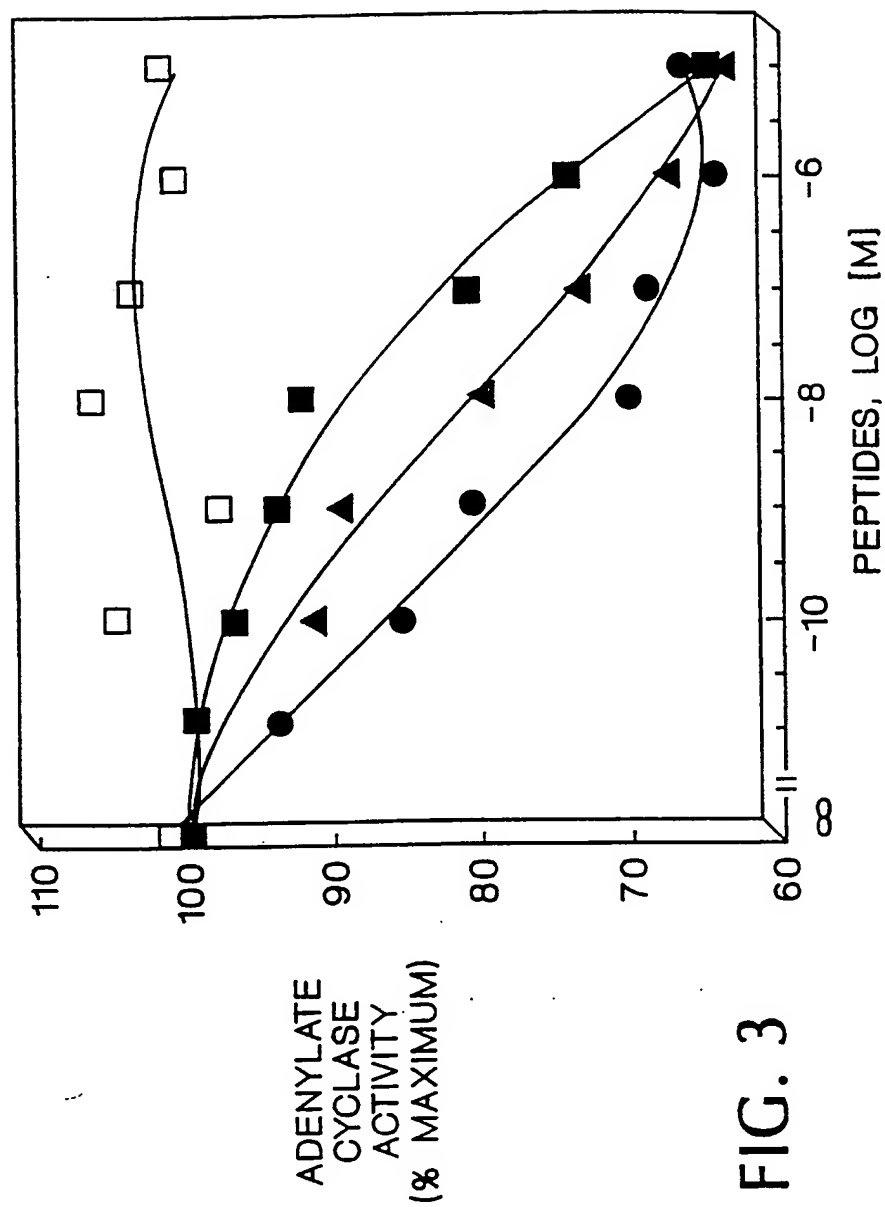


FIG. 3

4/12

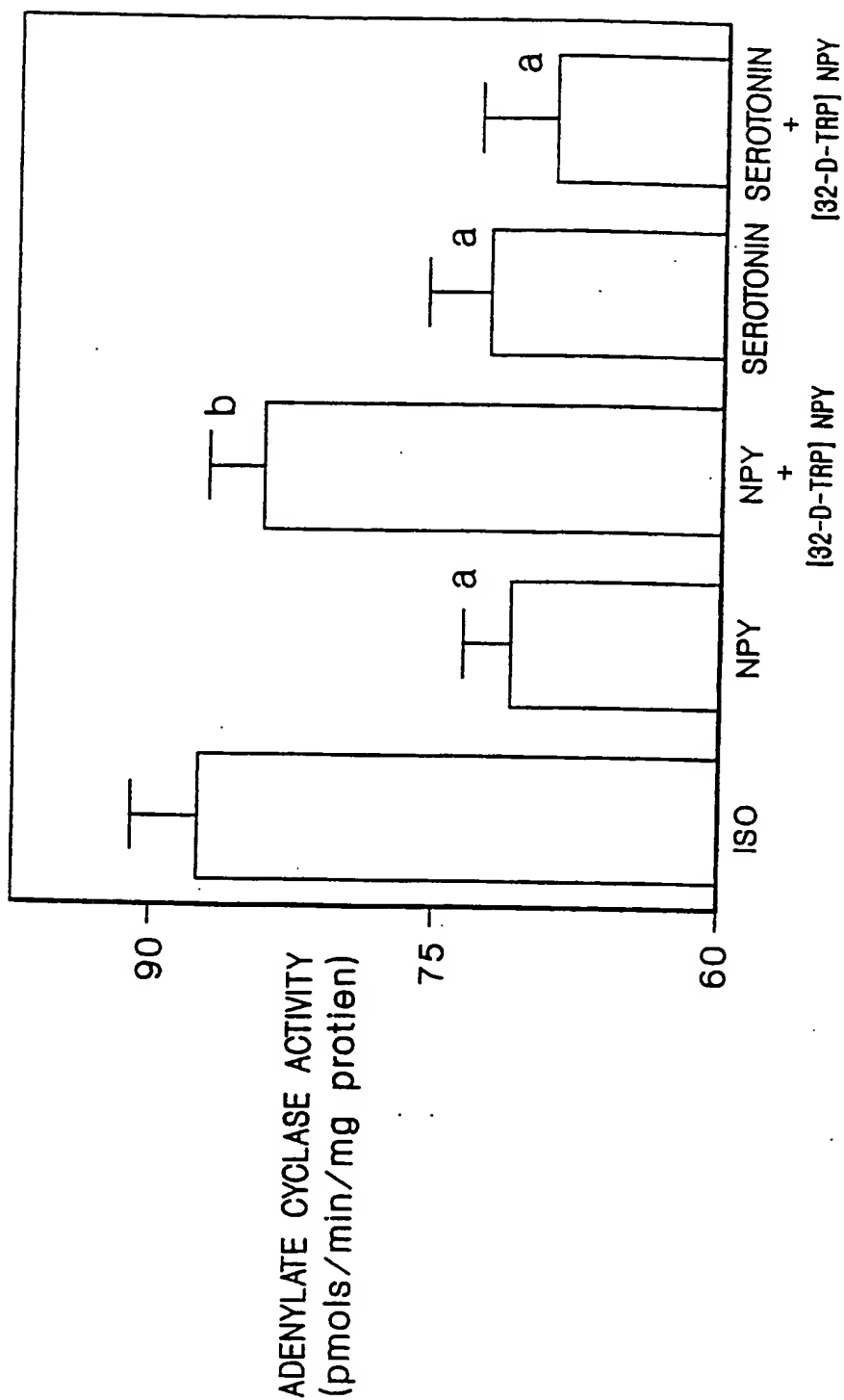


FIG. 4

5/12

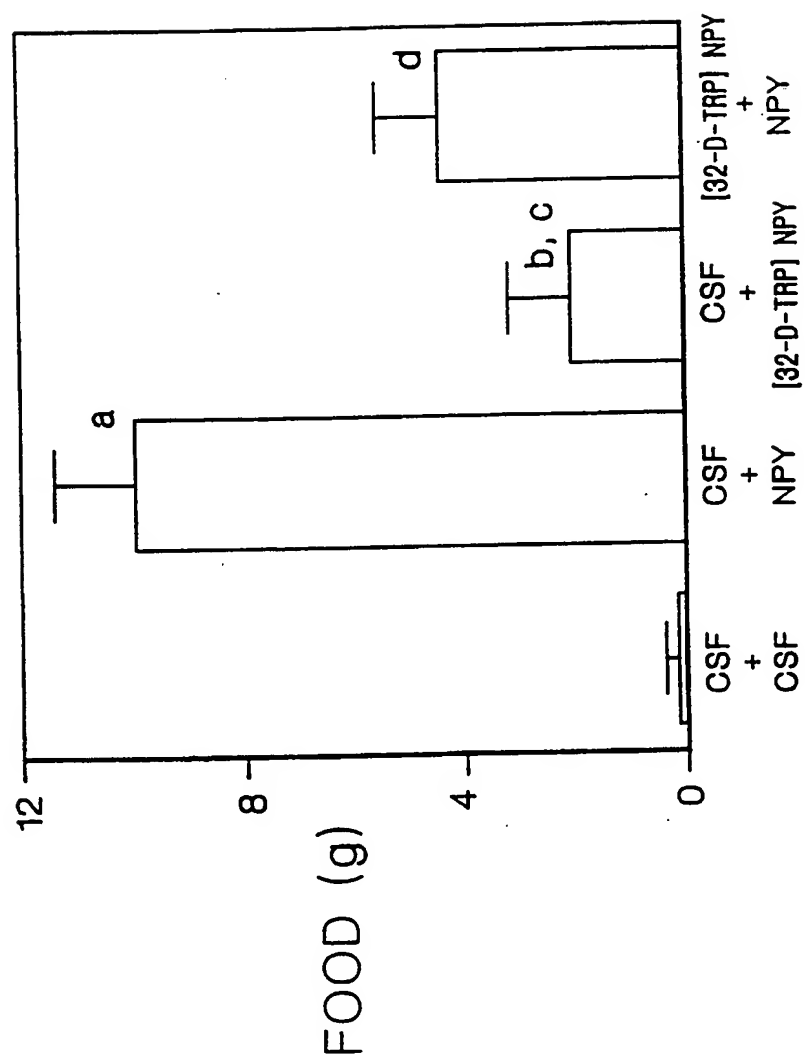


FIG. 5

6/12

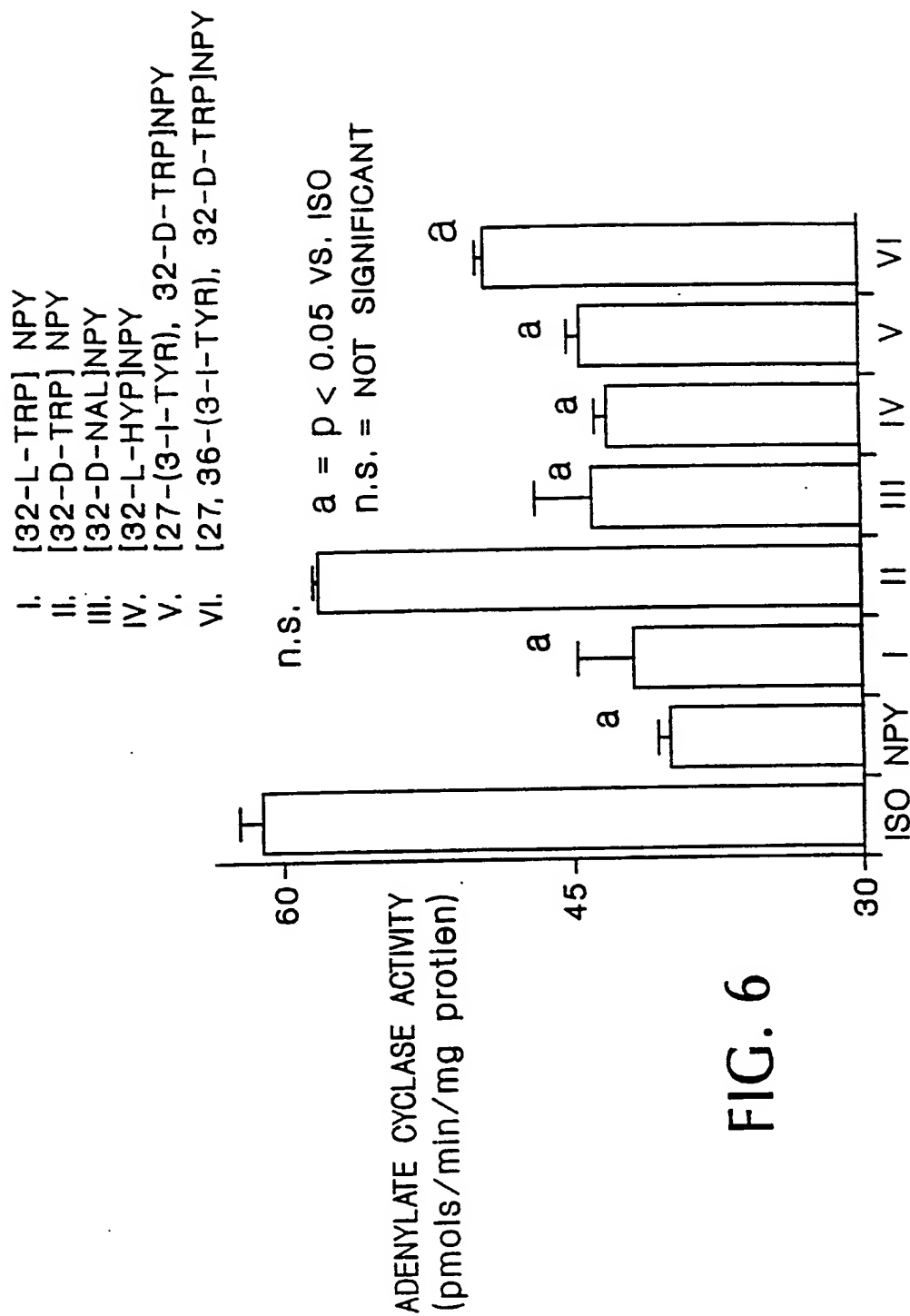


FIG. 6

7/12

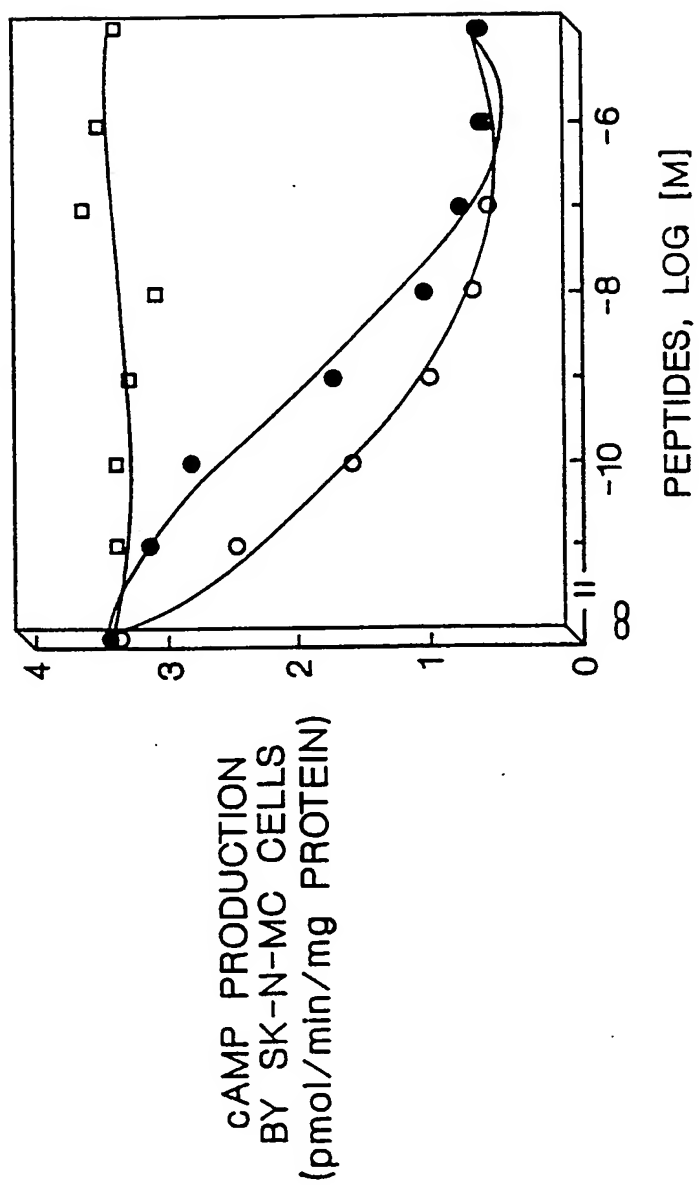


FIG. 7

8/12

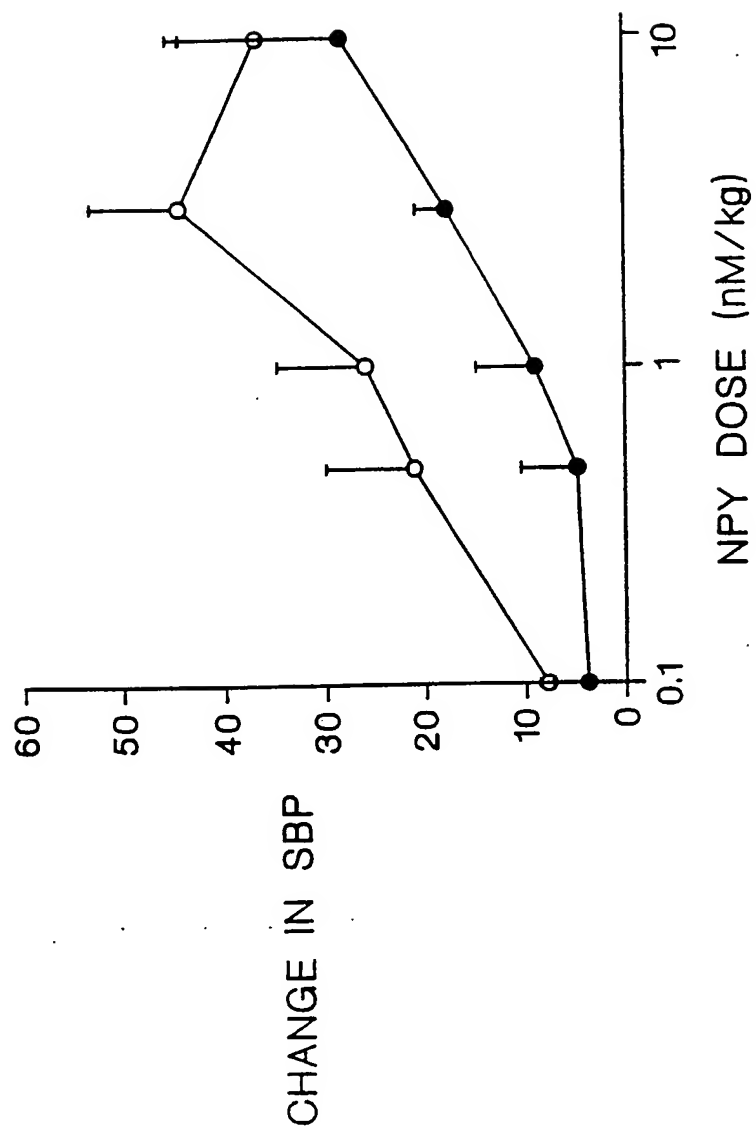


FIG. 8

9/12

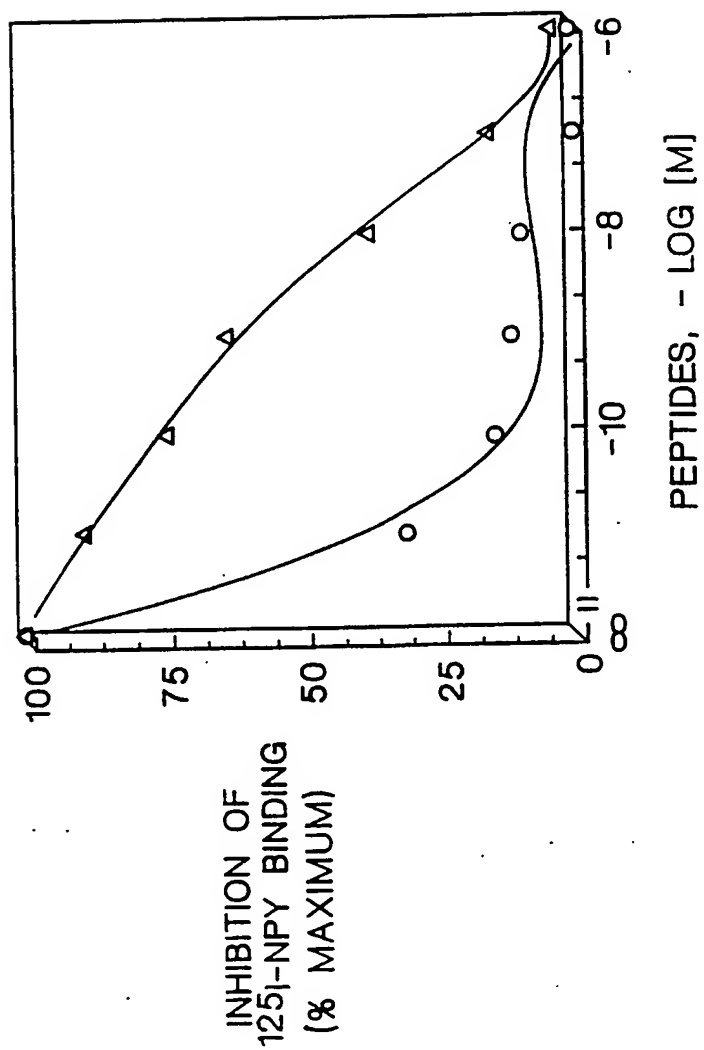


FIG. 9

10/12

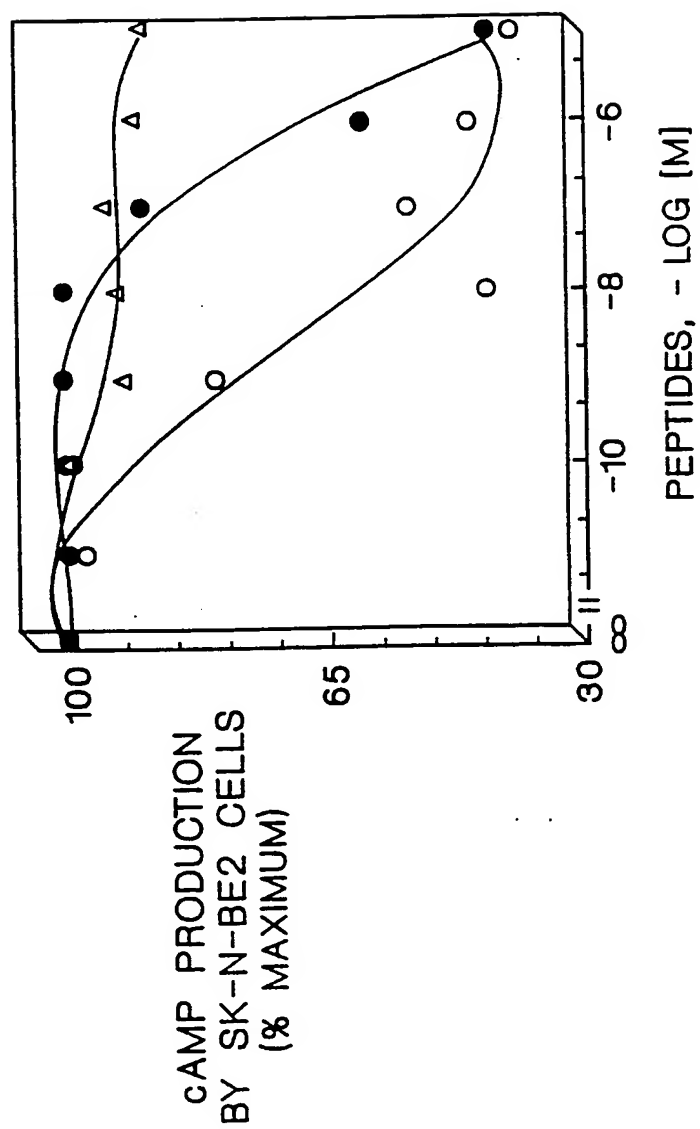


FIG. 10

11/12

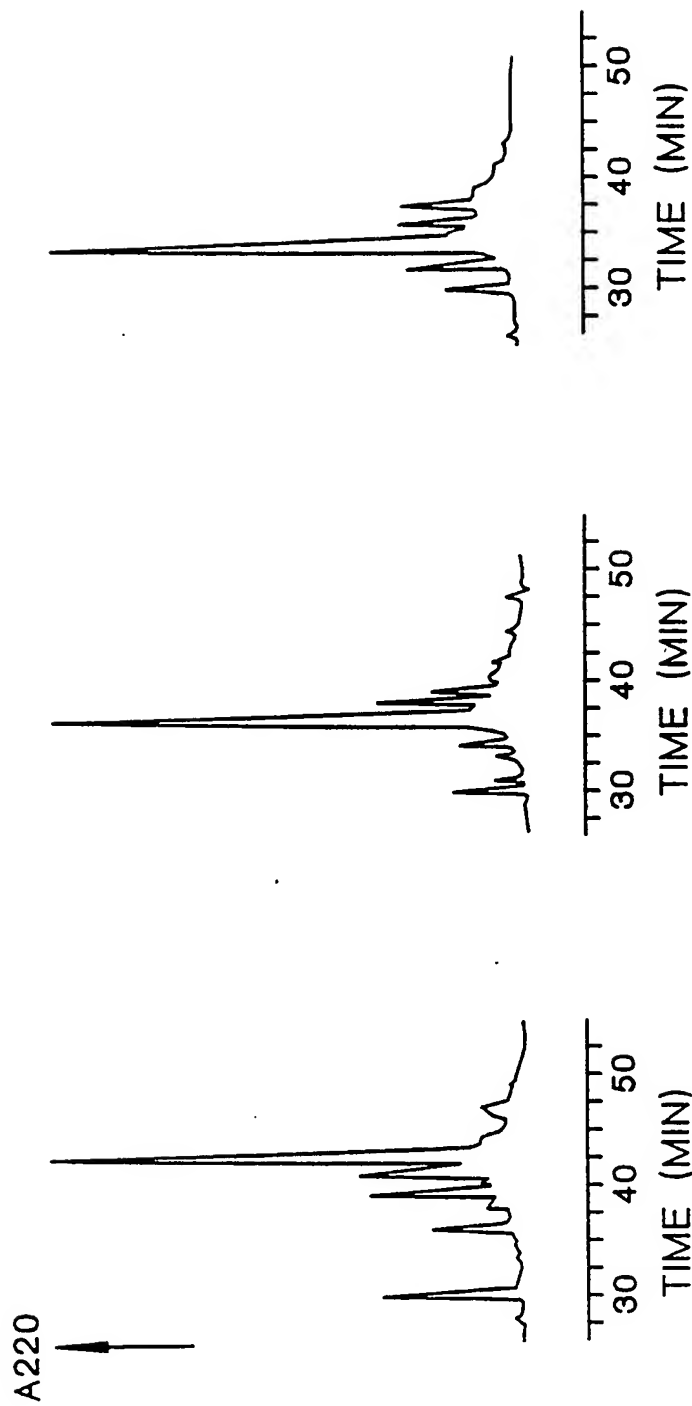


FIG. 11(c)

FIG. 11(b)

FIG. 11(a)

12/12

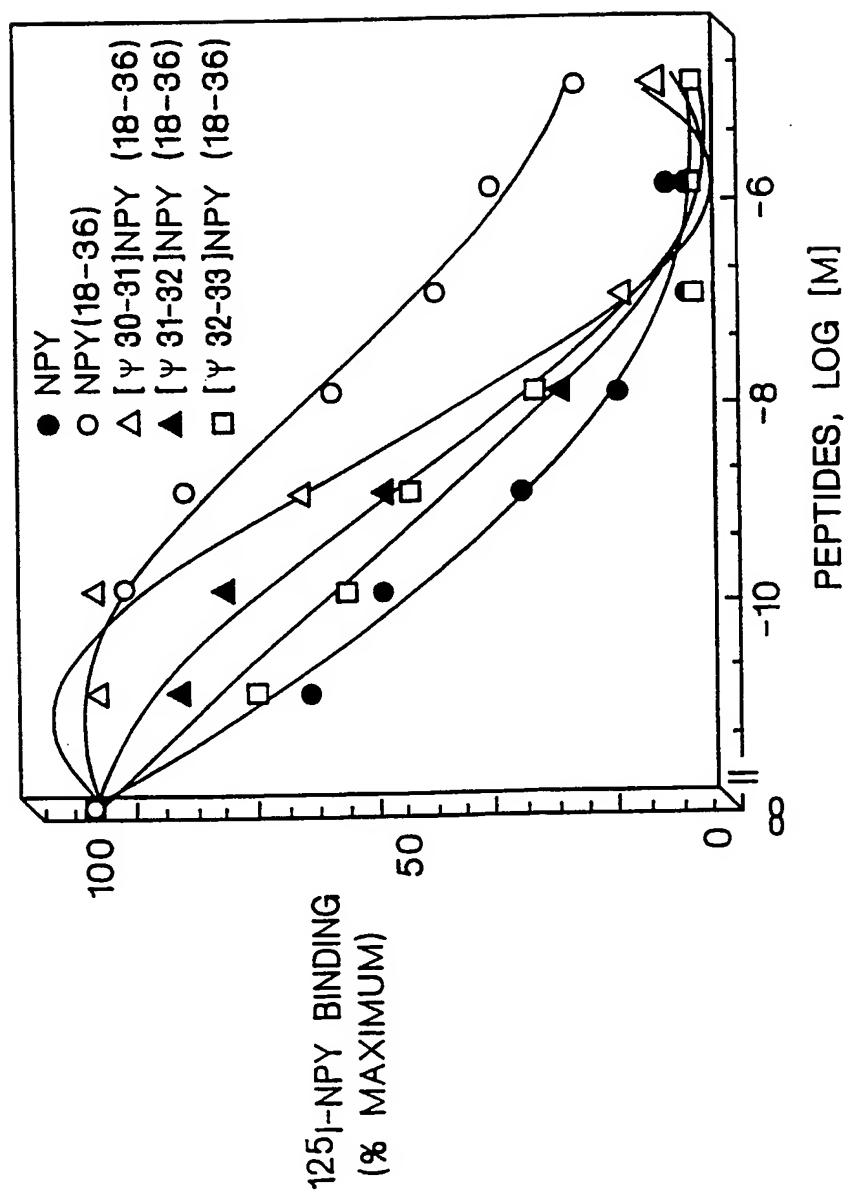


FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06837

A. CLASSIFICATION OF SUBJECT MATTER IPC(5) :A61K 37/02; C07K 5/00, 7/00, 15/00, 17/00 US CL :530/324, 325, 326 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 530/324, 325, 326 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) USPTO APS search terms: neuropeptide Y		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,026,685 (BOUBLIK ET AL) 25 June 1991, col. 3, lines 20-35.	5, 6, 12
X	US, A, 4,839,343 (WAEBER ET AL) 13 June 1989, col. 1, lines 20-60.	1,9
X	EP, A,0,355,793 (KRSTENANSKY ET AL) 28 February 1990, see p. 2.	1,9
X	J. Med. Chem., Volume 36, Number 3, issued 1993, D. A. Kirby et al, "Defining Structural Requirements for Neuropeptide Y Receptors Using Truncated and Conformationally Restricted Analogs", pages 385-393, see compounds #4,5 and 17.	1,2,9
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family
Date of the actual completion of the international search 08 AUGUST 1994		Date of mailing of the international search report AUG 18 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer SHEELA J. HUFF <i>Sheela Warden for</i> Telephone No. (703) 308-0916

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06837

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Med. Chem., Volume 35, issued 1992, R. D. Feinstein et al, "Structural Requirements for Neuropeptide Y18-36-Evoked Hypotension: A Systemic Study", pages 2836-2843, see compounds #1-3, 6-9, 12-15, 19-20, 22-27.	12
X	J. Med. Chem., Volume 32, issued 1989, Boublik et al, "Synthesis and Hypertensive Activity of Neuropeptide Y Fragments and Analogues with Modified N- or C-Termini or D-Substitutions", pages 597-601, see compounds 1-3, 5-6, 9, 11, 20-21, 23-24.	1
X	J. Med. Chem., Volume 36, issued 1993, D. A. Kirby et al, "Neuropeptide Y: Y1 and Y2 Affinities of the Complete Series of Analogues with Single D-Residues Substitutions", pages 3802-3808, see Table 1.	1

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.